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ORIGINAL ARTICLE

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In vivo antitumor efficacy of 17-DMAG (17-dimethylaminoethylamino-17-demethoxygeldanamycin hydrochloride), a water-soluble geldanamycin derivative

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Abstract *Purpose*: To describe the preclinical basis for further development of 17-dimethyl aminoethylamino-17-demethoxygeldanamycin hydrochloride (17-DMAG, NSC 707545). *Methods*: In vitro proliferation assays, and in vivo model studies in metastatic pancreatic carcinoma and subcutaneous xenograft melanoma and small-cell lung carcinoma models. *Results*: 17-DMAG emerged from screening studies as a potent geldanamycin analog, with the average concentration inhibiting the growth of the NCI anticancer cell line drug screen by 50% being 0.053 μM. "Head to head" comparison with 17-allylamino-17-demethoxygeldanamycin (17-AAG, NSC 330507) revealed 17-DMAG to possess potent activity against certain cell types, e.g., MDA-MB-231 breast carcinoma and HL60-TB leukemia which were relatively insensitive to 17-AAG. Evidence of oral bioavailability of 17-DMAG in a saline-based formulation prompted more detailed examination of its antitumor efficacy in vivo. 17-DMAG inhibited the growth of the AsPC-1 pancreatic carcinoma xenografts growing as intrahepatic metastases at doses of 6.7–10 mg/kg twice daily for 5 days administered orally under conditions where 17-AAG was without activity. 17-DMAG in an aqueous vehicle at 7.5–15 mg/kg per day for 3 days on days 1–3, 8–10 and 13–17, or 1–5 and 8–12 showed evidence of antitumor activity by the parenteral and oral routes in the MEXF 276 and MEXF 989 melanomas and by the parenteral route in the LXFA 629 and LXFS 650 adenocarcinoma and small-cell carcinoma models. The latter activity was comparable to the historical activity of 17-AAG. *Conclusions*: Taken together, the in vivo activity of 17-DMAG supports the further development of this water-soluble and potentially orally administrable geldanamycin congener.

Keywords Heat shock protein 90 · Ansamycin · Xenograft

Abbreviations AAALAC: Association for the Assessment and Accreditation of Laboratory Animal Care · 17-AAG: 17-Allylamino-17-demethoxygeldanamycin · 17-DMAG-HCl: 17-Dimethylaminoethylamino-17-demethoxy-geldanamaycin hydrochloride · GA: Geldanamycin · GI_{50}: Concentration of drug causing 50% growth inhibition · Hsp90: Heat shock protein-90 · IP: Intraperitoneal · IV: Intravenous · LC_{50}: Concentration of drug causing 50% cell kill · SC: Subcutaneous · T/C: Treated/control tumor weight · % T/C: growth delay · TGI: Concentration of drug causing total growth inhibition · USPHS: United States Public Health Service

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Introduction

Geldanamycin (GA) and related benzoquinone ansamycin agents were first isolated from the fermentation broth of *Streptomyces hygroscopicus* var. *geldanus* [8, 21]. GA and a number of analogs first became of interest as potential anticancer agents in the 1980s when a

member of this class, herbimycin, was found to "reverse" the transformed phenotype of v-*src*-transformed cells [29, 30]. The molecular basis for this phenomenon was revealed when GA and its analogs were found to be potent inhibitors of the heat shock protein Hsp90 co-chaperone function [27, 32]. V-*Src* and numerous other tyrosine kinase oncogenes require Hsp90 function to attain their properly folded active conformation and to arrive in the correct subcellular location. Without Hsp90 function these and numerous other client proteins including the protooncogenes, c-*erb*B2, c-*raf*1, alk, bcr-abl, akt, met and steroid hormone receptors [4, 5, 20, 25, 26, 31, 34] among others, become substrates for poly-ubiquitination and proteasome-mediated degradation. As many of these molecules play important roles in the regulation of cancer cell growth, the possibility of employing benzoquinoid ansamycins in cancer treatment is of considerable interest.

17-Allylamino-17-demethoxygeldanamycin (17-AAG, NSC 330507) is currently undergoing clinical trials both in the US and UK [22]. 17-AAG has actions with respect to Hsp90 essentially identical to those of GA in cells [23], although 17-AAG is less toxic to the host than related GAs [22, 28]. However, 17-AAG requires a complex formulation and forms a variety of metabolites, some potentially active or toxic [9]. This fact and concern that the agent's pharmaceutical properties may actually hinder clinical utility has prompted the search for more soluble analogs or novel chemotypes affecting Hsp90 function. 17-Dimethylaminoethylamino-17-demethoxygeldanamycin hydrochloride (17-DMAG, NSC 707545) is a water-soluble analog (11.2 mg/ml; R. Vishnuvajjala, personal communication) with highly attractive pharmaceutical properties [10]. 17-DMAG theoretically retains the capacity to bind Hsp90 [15], and it modulates Hsp90 client proteins in an essentially identical fashion to 17-AAG [24].

Efforts to complete preclinical evaluations of 17-DMAG and to expedite clinical trials are underway. We present here in vitro and initial in vivo data supporting the development of 17-DMAG as an antitumor agent with more broadly exploitable activity and more pharmaceutically tractable characteristics than 17-AAG. In particular, 17-DMAG in animal tumor models displays evidence of oral bioavailability, while 17-AAG does not. Recent studies [24] have provided evidence that the cellular pharmacology of 17-DMAG likewise potently recapitulates the essential desired features of the ansamycins.

Materials and methodologies

Compounds

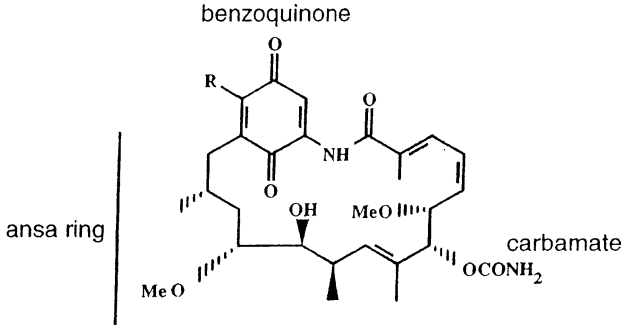
The chemical structures of 17-AAG (NSC 330507) and 17-DMAG (NSC 707545) are shown in Fig. 1. 17-DMAG was supplied by the NCI Developmental Therapeutics Program Drug Repository and is soluble in aqueous solution at greater than 10 mg/ml. For in vivo studies, 17-DMAG was dissolved in water or saline for oral administration and in saline or phosphate-buffered saline (PBS) for parenteral administration. The dosing solutions were freshly prepared every 1–3 days, and in efficacy studies the concentration was adjusted so that the administration volume was 10 ml/kg.

In vitro 60 cell line anticancer screen

The methods used for the NCI 60 cell line anticancer drug screen have been described elsewhere [18, 19]. Briefly, compounds are solubilized in dimethyl sulfoxide at ×200. The compounds are diluted into RPMI-1640 containing 5% fetal bovine serum (FBS) and serial 1-log dilutions are prepared for a total of five concentrations. Generally, the working range for initial testing of a compound is 10^{-4} through 10^{-8} *M*. Compounds are added to 24-h old cultures of each of the 60 cell lines used in the panel. Following a 48-h incubation, the medium is removed, cells are fixed and stained with sulforhodamine B, and total protein quantitated by optical methods. Through comparison with the amount of protein prior to drug addition (a time 0 control), the effect of a drug on cell growth can be estimated by calculating the drug concentration causing 50% growth inhibition (GI_{50}), total growth inhibition (TGI), and 50% cell kill (LC_{50}). These data are then plotted both as mean bar graphs and as dose-response curves. The similarity of cell susceptibility to the index and other compounds can be assessed by bioinformatics approaches such as the COMPARE algorithm as described previously [19].

In vitro time-course assay

Methods for cell culture, drug preparations, and conventional in vitro drug sensitivity testing have been



	NSC	R
Geldanamycin	122750	OMe
17-AAG	330507	$NHCH_2CH{=}CH_2$
17-DMAG	707545	$NHCH_2CH_2NMe_2$

Fig. 1 Chemical structure of GA, 17-allylamino-17-demethoxygeldanamycin (NSC 330507) and 17-dimethyl aminoethylamino-demethoxygeldanamycin HCl (NSC 707545)

described previously [1]. The cell lines and concentration ranges of experimental agents to be evaluated in the concentration–time (c×t) assays were chosen on the basis of 60-cell line screening data and from the results of hollow fiber in vivo assays. The selected cell lines included the human promyelocytic leukemia, HL-60 (TB), a human melanoma LOX-IMVI, and an estrogen-independent human breast cancer MDA-MB-231. As described elsewhere [2], exposure to an experimental agent for increasing periods of time, followed by drug removal, permits quantitation of drug activity conferred by each of several exposure durations ranging from ≤1 h to 96 h. Comparison with plates not exposed to drug permits determination of the GI_{50}, TGI, and LC_{50} endpoints. From the plotting of composite c×t data, the minimum exposure conditions (both concentration and time) required to achieve cytostatic and/or cytocidal activity in a given cell line can be readily determined, and the relative sensitivities of multiple cell lines can then be compared to identify the most sensitive cell types for in vivo efficacy evaluations in xenograft models.

Hollow fiber assay

Cells loaded into semipermeable polyvinylidene fluoride hollow fibers can be implanted into the subcutaneous (SC) and intraperitoneal (IP) body compartments of mice that subsequently receive the test compound. This allows preliminary in vivo efficacy evaluations as a prelude to more detailed evaluation in xenograft models. 17-DMAG was evaluated in the hollow fiber assay as described previously [13, 14]. 17-DMAG was prepared for dosing in sterile water or saline and treatment was started on the 3rd or 4th day after hollow fiber implantation. On day 7 or 8, the fibers were collected and the viable cell mass determined using a formazan dye conversion assay [2]. The viable cell mass in the treated fibers was compared to that in the vehicle treated fibers, and each time the treated group had a 50% or greater reduction in viable cell mass compared to control, it was assigned a score of 2 [14]. The total scores were determined by summing the IP and SC values obtained against each cell line implanted at both dose levels in both of the respective body compartments. The maximum possible score is 96 since there are a total of 48 combinations of cell lines, doses and implantation sites. Based on prior hollow fiber experience, a score of 20 or greater correlates with increased likelihood of activity in conventional SC xenograft models [14, 16].

Orthotopic liver metastasis model

Since the liver is a known target organ for toxicity of the GAs [22, 28], the initial xenograft model evaluated was an orthotopic model in which the tumor developed in the liver parenchyma. For this, AsPC-1 human pancreatic tumor cells were injected into the spleen of anesthetized male athymic (NCr nu/nu) mice followed by a complete splenectomy 10 min after injection. The tumor cells, distributed from the spleen prior to splenectomy, established multifocal tumors within the liver of the host animals with a tumor take rate of 100%. The presence of tumor in the liver was readily visualized 30–40 days after injection, and the day the animals were killed was selected by verifying significant increases in liver mass in the control mice through palpation. The tumor mass was quantitated by recording the total liver weight for each experimental animal on the day the animals were killed. Three separate experiments were conducted using this model with treatments administered by the oral route using twice-daily dosing for a minimum of 5 to a maximum of 20 days. For each experiment there were 15–20 mice in the control group and 9 or 10 in the treated groups. The liver weights of each treated group were compared with those of the control group using Student's *t*-test with significance set at 0.05. These experiments were performed in compliance with the USPHS Guidelines for Humane Animal Care and Use in an AAALAC-approved facility.

Assessment of in vivo activity of 17-DMAG-HCl in human melanoma and lung cancer SC xenografts

17-DMAG was tested for in vivo activity in four melanoma models using the Freiburg human tumor xenograft panel including MEXF 276, MEXF 462, MEXF 514, and MEXF 989 as well as two lung xenografts, namely the LXFA 629 lung adenocarcinoma and the LXFS 650 small-cell lung carcinoma. The xenografts were initially derived from patient surgical specimens and directly implanted into nude mice. The tumors were then propagated until stable growth occurred and master stocks were frozen in liquid nitrogen in early passages. A particular master stock batch itself is only used for about ten further passages. Therefore, these xenografts closely reflected the initial primary tumor histology. Establishment and characteristics of the models have been described previously [6, 11]. These studies were performed in accordance with the German Animal Protection Act and project license regulations identical to those of the United Kingdom Coordinating Committee on Cancer Research Guidelines for the Welfare of Animals in Experimental Neoplasia.

Nu/nu athymic mice of NMRI background from an in-house breeding facility were used for all experiments. Tumors were implanted SC in both flanks of 6-week-old mice. Treatment was initiated when tumors reached a volume of 80–200 mm^3 depending on the growth behavior of the xenograft model. Animals were randomly assigned to treatment groups. Food and water were provided ad libitum. Tumor growth was followed by serial two-dimensional caliper measurements and body weight documented concomitantly twice a week. Tumor volumes were calculated according to the formula (tumor length×tumor $width^2$)/2 and mean as well

as median relative tumor volume [(volume day x/volume day 0)×100] were used for all further analyses. Data are presented as mean relative tumor volumes, mean standard error, optimal T/C and growth delay 400% as detailed previously [6]. Drug doses and treatment schedules were delineated from the determination of a maximum tolerated dose (MTD) in nontumor-bearing nude mice prior to the start of the xenograft experiments (see Table 1). The in vivo experiments were performed twice and representative data are shown. The Wilcoxon test was used to determine statistical significance of each treatment versus control data. Systat version 10 (SPSS, 2000) was used to perform statistical analyses.

Results

In vitro antitumor activity profiles of 17-AAG and 17-DMAG

17-AAG (NSC 330507) and 17-DMAG (NSC 707545) each exhibit multi-log differential patterns of activity in the Developmental Therapeutics Program in vitro cancer screen, with certain cell types very sensitive, and others somewhat resistant, to the drug. Typical average mean graph patterns of activity in the standard 2-day assay for 17-DMAG (48 h "continuous" drug exposure) are shown in Fig. 2. In this representation, the mean GI_{50} (0.053 μM), TGI (6.3 μM) or 50% kill (LC_{50}, 44 μM) is plotted as the middle line, and the behavior of each cell line is plotted as a bar deflecting by convention to the right for cells more sensitive than the mean and to the left for cells more resistant than the mean. A range of 1- to 2.63-log difference in sensitivity with respect to the means is apparent at the GI_{50} level of growth inhibition. In COMPARE analysis against the synthetic agent database, 17-AAG was the compound most closely correlated with 17-DMAG. Profiles for the two agents had a Pearson correlation coefficient (PCC) of 0.783 at the GI_{50} level and a PCC of 0.668 at the TGI level (data not shown).

Assessment of in vitro concentration×time activity

To define a minimum target concentration, and time of exposure for further preclinical or clinical studies, the in vitro time-course assays of a subset of sensitive as well as insensitive cell lines were examined. These studies demonstrated consistently greater activity of 17-DMAG than of 17-AAG. While sensitive cell lines (e.g. HL 60(TB)) responded to brief exposures (<1 h) of less than 1 μM, the best activities (minimum c×t values) accompany longer drug exposures (generally >12 h, with some cell lines requiring >48 h) to <0.1 μM to achieve drug-induced lethalities (Fig. 3).

Of interest, certain cell lines which were observed to be relatively insensitive to 17-AAG (e.g., MDA-MB-231 and HL-60) were far more sensitive to 17-DMAG. As shown in Fig. 3, even brief exposures to the latter (<1 μM for 1–3 h) are capable of conferring not only cytostatic activity (GI_{50} and TGI levels) but also cytocidal activity (LC_{50}). Nevertheless, as with 17-AAG, the best activities (minimum c×t values) for the 17-DMAG analogs were observed for the longer exposure durations.

"Drug stability" time-course assays of 17-AAG and 17-DMAG were also conducted as part of the in vitro assessment of these agents. These experiments (in which solubilized drugs are "preincubated" at 37°C for up to 48 h prior to addition to cultured cells) demonstrated that the anticancer activity of each of these agents is stable in vehicle as well as in culture medium for at least 48 h (data not shown).

Hollow fiber assay

Based upon single administration MTD determinations of 17-DMAG, the doses evaluated were 75 and 50 mg/kg daily for 4 days by the IP route and 37.5 and 25 mg/kg daily for 4 days by oral gavage to mice bearing fibers loaded with 12 cell lines selected from the in vitro screen. Following IP dosing, 17-DMAG produced an IP score of 24 and a SC score of 24 for a total score of 48 out of a maximum total possible score of 96 (data not shown). Similarly, oral dosing in the hollow fiber assay resulted in an IP score of 24 and a SC score of 18, for a total score of 42 (data not shown). In contrast to these results,

Fig. 2 GI_{50}, TGI, and LC_{50} mean graphs for 17-DMAG (NSC 707545). Response parameter definitions, methods of construction and interpretation of mean graphs are summarized in the Methods section with analytical details described elsewhere [18]. These mean-graph "fingerprints" can be shown by pattern-recognition analyses (i.e., COMPARE) to be highly correlated with agents of the same chemical class which share similar mechanism(s) of action

Table 1 Dose-finding study for 17-DMAG in NMRI nu/nu mice

Treatment	Dose (mg/kg/dose)	Schedule (day)	Route	Drug-related deaths	Maximal body weight change (%)
Control[a]		1–3, 6–8	IP	0/2	+2
17-DMAG	30	1–3, 6–8[b]	IP	0/2	−23
	30	1–3, 6–9[b]	Oral	1/2	−14
	15	1–3, 6–9	IV	0/2	−13

[a]Saline 10 ml/kg
[b]Total daily dose of 30 mg/kg was split into two injections of 15 mg/kg given 7 h apart

National Cancer Institute Developmental Therapeutics Program
Mean Graphs

NSC: S 707545	Units: Molar	SSPL: r	Exp. ID: Averaged
Report Date: June 4, 2001		HC -4.0 TT 08:	

Panel/Cell Line	Log_{10} GI50	Log_{10} TGI	Log_{10} LC50
Leukemia			
CCRF-CEM	-7.60	-4.96	-4.00
HL-60(TB)	-7.14	-6.14	-4.38
K-562	-7.85	-4.82	-4.08
MOLT-4	-7.52	-5.06	-4.11
RPMI-8226	-7.15	-5.39	-4.19
SR	-8.00	-5.07	-4.02
Non-Small Cell Lung Cancer			
A549/ATCC	-8.00	-4.72	-4.05
EKVX	-6.77	-5.08	-4.47
HOP-62	-7.00	-4.72	-4.27
HOP-92	-6.68	-5.62	-4.15
NCI-H226	-6.34	-4.92	-4.26
NCI-H23	-8.00	-5.11	-4.26
NCI-H322M	-5.85	-4.80	-4.25
NCI-H460	-8.00	-5.40	-4.33
NCI-H522	-7.70	-5.48	-4.41
Colon Cancer			
COLO 205	-7.85	-6.60	-5.04
HCC-2998	-7.82	-6.04	-4.89
HCT-116	-7.77	-5.60	-4.66
HCT-15	-6.21	-4.96	-4.19
HT29	-7.60	-5.38	-4.20
KM12	-7.92	-6.48	-4.33
SW-620	-7.26	-4.46	-4.00
CNS Cancer			
SF-268	-6.62	-4.74	-4.10
SF-295	-7.54	-4.92	-4.42
SF-539	-7.60	-5.44	-4.51
SNB-19	-7.54	-4.80	-4.33
SNB-75	-8.00	-5.32	-4.36
U251	-7.96	-5.11	-4.44
Melanoma			
LOX IMVI	-7.96	-6.89	-4.46
MALME-3M	-7.89	-6.07	-5.11
M14	-8.00	-5.20	-4.52
SK-MEL-2	-7.12	-4.92	-4.30
SK-MEL-28	-6.46	-4.43	-4.14
SK-MEL-5	-7.66	-5.96	-5.19
UACC-257	-7.80	-5.92	-5.18
UACC-62	-7.77	-5.28	-4.59
Ovarian Cancer			
IGROV1	-7.85	-5.68	-4.60
OVCAR-3	-7.47	-5.62	-4.43
OVCAR-4	-5.96	-5.29	-4.77
OVCAR-5	-6.92	-5.12	-4.28
OVCAR-8	-7.77	-5.16	-4.42
SK-OV-3	-7.85	-4.96	-4.11
Renal Cancer			
786-0	-7.54	-4.62	-4.09
A498	-4.89	-4.54	-4.19
ACHN	-7.21	-4.64	-4.11
CAKI-1	-5.85	-4.39	-4.00
RXF-393	-7.51	-5.05	-4.33
SN12C	-7.96	-5.38	-4.54
TK-10	-7.64	-4.47	-4.04
UO-31	-6.08	-4.49	-4.07
Prostate Cancer			
PC-3	-7.66	-4.80	-4.08
DU-145	-7.57	-4.85	-4.38
Breast Cancer			
MCF7	-8.00	-5.34	-4.48
MCF7/ADR-RES	-4.72	-4.19	-4.03
MDA-MB-231/ATCC	-7.82	-5.70	-4.21
HS 578T	-5.48	-4.19	-4.02
MDA-MB-435	-8.00	-5.89	-5.32
MDA-N	-8.00	-5.66	-4.52
BT-549	-5.68	-4.68	-4.29
T-47D	-7.21	-5.28	-4.23
MG_MID	-7.28	-5.20	-4.36
Delta	0.72	1.69	0.96
Range	3.28	2.70	1.32

17-AAG produced a total score of 38 following IP administration but a total score of only 6 following oral administration (data not shown). These results are highly suggestive of potential antitumor activity for 17-DMAG in conventional xenograft models [14, 16] and support the selection of the oral route of administration

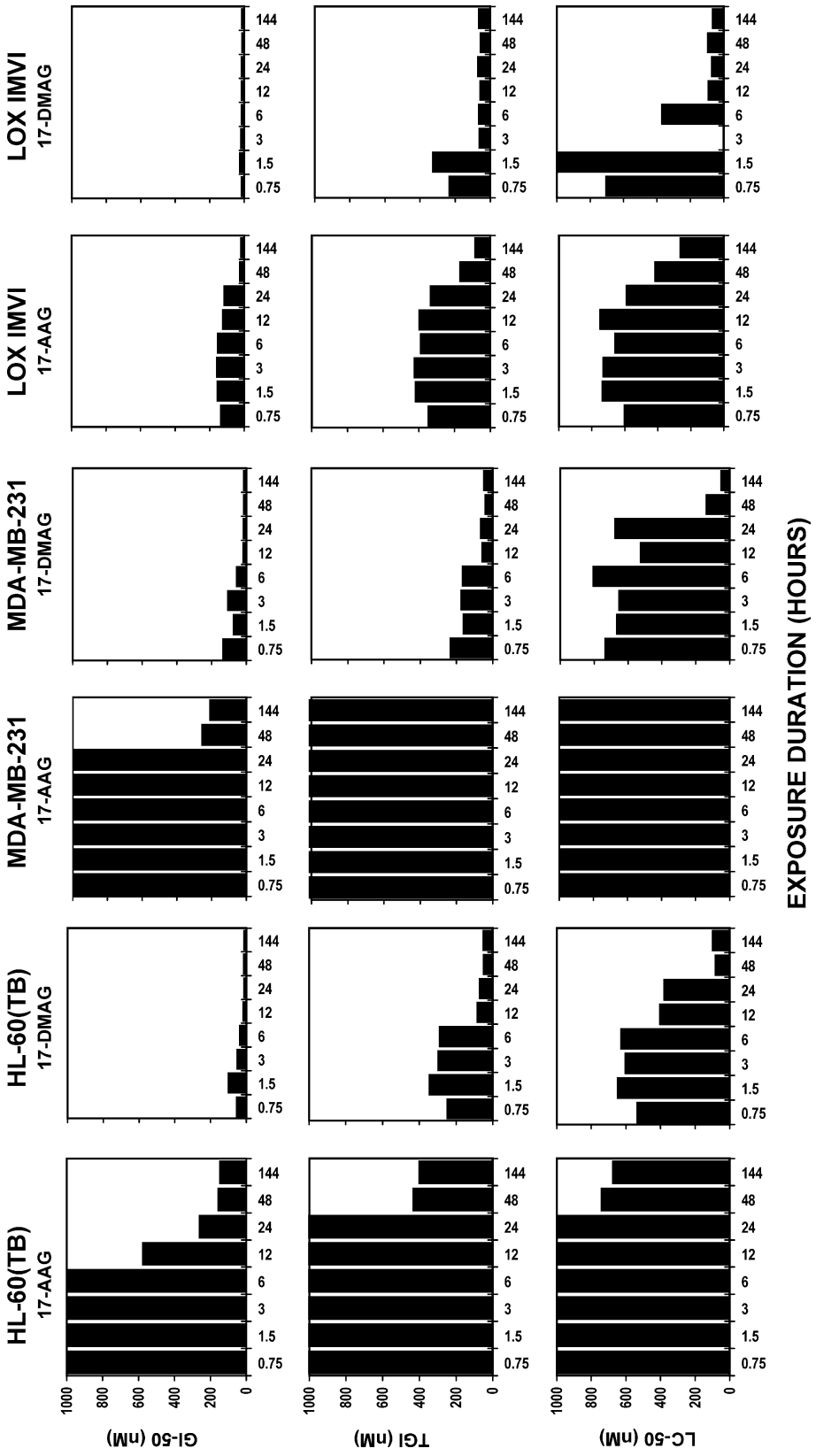


Fig. 3 Comparative in vitro concentration×time activity profiles of 17-AAG (NSC 330507) and 17-DMAG (NSC 707545) in three sensitive tumor cell lines. The concentrations (*ordinate*) and durations of exposure (*abscissa*) of drug required to achieve GI_{50}, TGI and LC_{50} are shown for each of three cell lines: HL-60 (TB) (promyelocytic leukemia), MDA-MB-231 (breast carcinoma), and LOX-IMVI (melanoma). These composite sets of bar graphs may allow estimation of the minimum concentration×time exposure requirements for each cell line, and allow estimation of the relative sensitivity of the cell lines

for further efficacy studies. Note that as the hollow fiber assay is a short-term screening assay extending for only 8 days of observation, doses used in the hollow fiber assay are frequently higher than might be possible over more extended dosing schedules.

Intrahepatic human pancreatic tumor xenografts

The initial xenograft model explored the AsPC-1 human pancreatic carcinoma model growing as a metastatic intrahepatic model. Following drug exposure, this model can be scored as the liver weight in treated as compared to control animals. The initial in vivo xenograft experiment conducted with 17-DMAG used oral dosing at 50, 33.4 and 22.2 mg/kg per dose given twice daily 5 days per week for the duration of the experiment. These doses were selected based upon the original single mouse toxicity results and the hollow fiber assay. On a chronic dosing schedule, these doses resulted in significant toxicity. A subsequent experiment used oral dosing at 15, 10 and 6.7 mg/kg per dose given twice daily 5 days per week for 4 weeks starting on day 5 after tumor implantation with the animals killed and the tissues collected on day 36. The 15 mg/kg twice daily dose was associated with mortality in 30% of the treated mice. There was a significant reduction in total tumor burden at all three dose levels (Fig. 4a) evaluated including the mid-dose and low dose which were not associated with lethality, nor with significant body weight loss compared with controls (Fig. 4b). As the age-related liver weight of nontumor-bearing mice is approximately 0.9 g, the drug effect, while clearly significant, did not result in tumor elimination in these animals, where treatment was initiated 5 days after tumor cell injection.

A direct comparison with 17-AAG was undertaken, using 15, 10 and 6.7 mg/kg per dose of 17-DMAG given twice daily 5 days per week for 4 weeks as was done in the preceding experiment; however, in this experiment treatment was initiated on day 2 rather than day 5. 17-AAG was included in this experiment using the oral route of administration, at 50 mg/kg per dose twice daily on the same schedule as 17-DMAG. As shown in Fig. 5a, there was a marked decrease in liver tumor burden at all doses of 17-DMAG while 17-AAG administered orally did not produce a reduction in tumor burden even at a dose that was more than five times higher than the effective dose of 17-DMAG on a milligram per kilogram basis. At 15 mg/kg per dose twice daily, 17-DMAG resulted in mortality in seven of the ten mice so that 10 mg/kg per dose twice daily on a chronic schedule (repeated cycles of daily for 5 days) appears to be the MTD by the oral route (Fig. 5b).

Maximum tolerated dose for SC xenograft studies

To expand the information available regarding dose and route effects, parenteral routes of administration

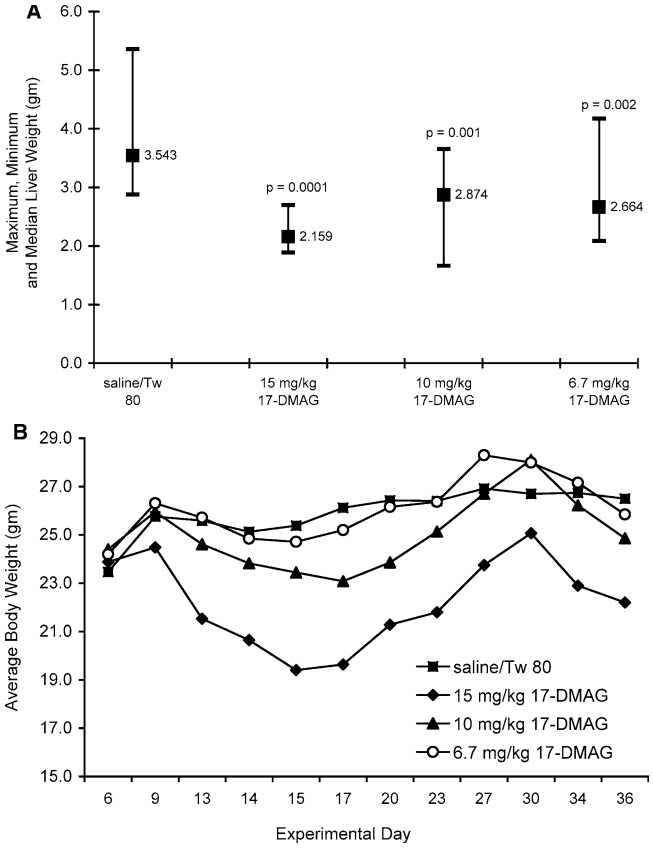


Fig. 4 Activity by the oral route in the metastatic AsPC-1 pancreatic carcinoma model. 17-DMAG was administered orally at doses of 15, 10 and 6.7 mg/kg per dose given twice daily for 5 days followed by a 2-day rest with the cycle repeated a total of four times with the first treatment given on day 5 after tumor implantation. The mice were killed on day 36 and their liver weights were recorded. The maximum, minimum and median liver weights for each group (**a**) and the group average body weights (**b**) are shown.

were investigated. The IV MTD for 17-DMAG was established at 15 mg/kg per day when given once-daily for 3 days for 2 weeks in nontumor-bearing NMRI nu/nu mice (Table 1). At this dose and schedule, 17-DMAG was not lethal but produced a body weight loss of 13% compared to the starting weight. Experiments with tumor-bearing mice showed that the treatment cycles could be repeated up to three or four times and extended to a daily for 5 days schedule. The initial oral dose was 30 mg/kg per day given as two equal doses 7 h apart. This dose proved toxic in that one of two mice died accompanied by a weight loss of 14% (Table 1). For chronic oral treatment on a daily for 5 days schedule, the selected nontoxic dose was 15 mg/kg. The initial IP dose was also 30 mg/kg per day daily for 3 days given in two doses at 0 and 7 h, but this produced severe body weight loss (24%). IP doses of 30 and 20 mg/kg per day in tumor-bearing mice resulted in 23% toxic deaths. The IP route was therefore abandoned for SC xenografts (data not shown).

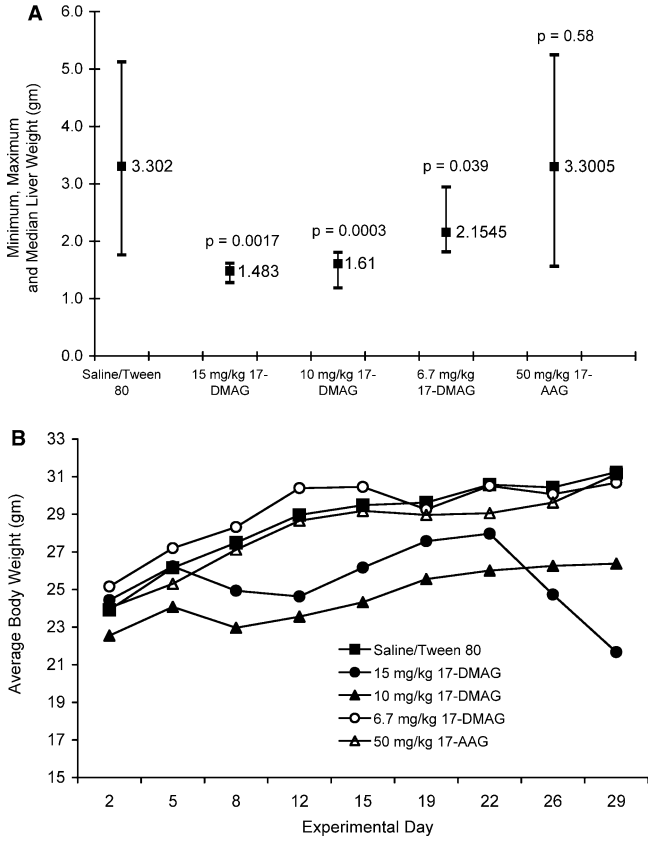


Fig. 5 Comparison of 17-DMAG and 17-AAG in the metastatic AsPC-1 pancreatic carcinoma model. 17-DMAG was administered orally at 15, 10 and 6.7 mg/kg per dose twice daily 5 days per week (four cycles) with treatments starting on day 2 and the animals killed on day 30. In this experiment, 17-AAG was also evaluated on the same schedule at 50 mg/kg per dose given orally. The maximum, minimum and median liver weights for each group (**a**) and the group average body weights (**b**) are shown.

17-DMAG efficacy in human tumor xenografts

Human xenograft models for in vivo efficacy studies were chosen based on their in vitro sensitivities and on their known responsiveness to 17-AAG [7, 24]. The four melanoma xenografts MEXF 276, MEXF 462, MEXF 514 and MEXF 989 and the two lung carcinomas LXFA 629 and LXFS 650 were selected [6, 7]. The melanomas MEXF 989 (Fig. 6a) and MEXF 276 (Fig. 6b) proved to be sensitive to 17-DMAG-HCl, the melanomas MEXF 514 (Fig. 6c) and MEXF 462 (data not shown) were found to be "resistant" to the drug in terms of optimal test/control results. 17-DMAG-HCl was highly active if given IV at 15 mg/kg per dose in MEXF 276 with a T/C of 12% ($P < 0.043$) and in MEXF 989 with a T/C of 3% ($P < 0.018$). A clear dose-response relationship was seen in the MEXF 276 model between the doses of 7.5 and 15 mg/kg per day (Fig. 6B, Table 2). Given orally in the responsive MEXF 276 model, 17-DMAG proved to be bioavailable and was markedly active with a T/C of 27% ($P < 0.043$; Table 2, Fig. 6B).

In contrast, the melanotic melanoma MEXF 514 (Fig. 6C) and the amelanotic MEXF 462 xenografts (not shown) were relatively "resistant" to 17-DMAG treatment. At 15 mg/kg, MEXF 514 showed a T/C of 70% and MEXF 462 a T/C of 66%. The effects of 17-DMAG in MEXF 514, however, were statistically significant despite a marginal tumor inhibition of only 30% (Table 2). The two lung cancer xenografts studied, LXFA 629 and LXFS 650 (Table 2, Fig. 6D, respectively), exhibited similar in vivo activity (T/C values of about 50%).

Discussion

In the study reported here we demonstrated that 17-DMAG possesses more potent antiproliferative activity in vitro than 17-AAG, yet the pattern of cell sensitivity to the agent suggests that the mechanisms of action of 17-DMAG and 17-AAG are similar. Smith et al. [24] have documented comparable downregulation of Hsp90 client molecules. We demonstrated further that 17-DMAG possesses antitumor activity when administered by the oral route, in contrast to 17-AAG, in orthotopic and SC models of pancreatic cancer and melanoma, and possesses at least comparable or somewhat better activity in comparison to 17-AAG when administered parenterally to animals bearing SC melanoma and lung carcinoma xenografts.

17-DMAG's greater potency in vitro is mirrored by evidence of a somewhat greater potential for toxicity in vivo, as the MTDs of 17-DMAG in several administration regimens appeared to be lower than those observed for 17-AAG. We recognize that this finding may portend a lower MTD when 17-DMAG is used in human clinical trials. However, several pieces of evidence suggest that clinical evaluation of 17-DMAG may be warranted. First, clear evidence of an antitumor effect was observed by the oral route. This opens the possibility of chronic administration schedules that may be able to achieve relatively low concentrations for protracted intervals, a clinical goal not feasible with 17-AAG. Second, in studies discussed elsewhere [10], metabolism and protein binding of 17-DMAG appear to be intrinsically lower and less avid, respectively, than those of 17-AAG, and therefore the possibility of more facile distribution to intratumoral sites of a single active drug substance may be possible with 17-DMAG. Third, as demonstrated here, noteworthy antiproliferative effects are possible using an aqueous (saline based) vehicle, a marked advantage of 17-DMAG as early clinical experiences with 17-AAG [22] have suggested that at total doses > 300 mg/m^2 the physical composition of the DMSO/egg phospholipid vehicle becomes unwieldy and may actually limit further clinical exploration of dose. That humans could tolerate such doses was surprising. An intrinsically more potent GA congener would promote further dose escalation efforts at infrequent

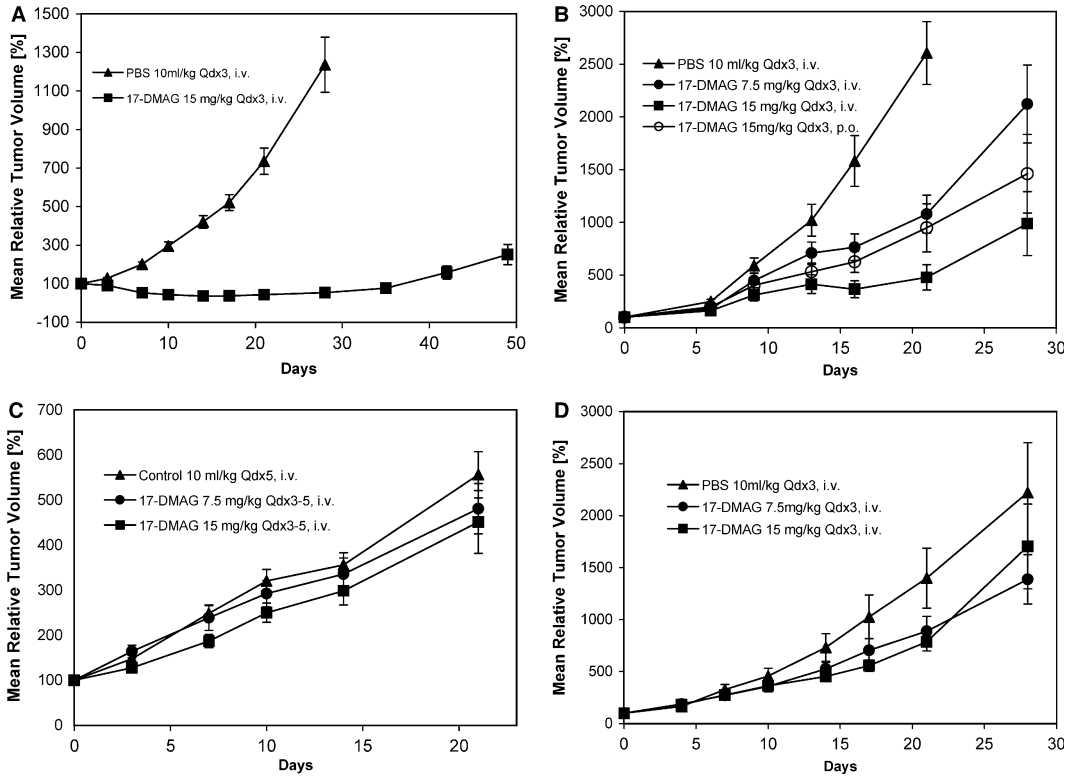


Fig. 6 Activity of 17-DMAG in conventional human xenografts growing SC in nude mice (mean ± SEM relative tumor volumes are shown): **A** melanoma MEXF 989; **B** melanoma MEXF 276; **C** melanoma MEXF 514; **D** small-cell lung cancer LXFS 650 (mean relative tumor volumes on day 0, the day of randomization: 180.2 ± 7, 110 ± 15.7, 100.6 ± 8, and 88.3 ± 13.6 mm^3, respectively). Treatment, route, dose and schedule are shown in the figures

intervals of administration. Thus, whether the clinical goal is long-term exposure to low concentrations or intermittent administration of relatively higher doses, 17-DMAG appears to offer superior opportunities provided its tolerability in humans is acceptable, which will require phase I clinical evaluation.

The basis for 17-DMAG's greater potency is not clear. While it theoretically can clearly bind to Hsp90 in a way that recapitulates aspects of the binding of 17-AAG [15], and modulate Hsp90-associated client proteins [24], formal measurement of the affinity of 17-DMAG for Hsp90 has not yet been completed. Indeed, recent evidence provided by Kamal et al. [17] is consistent with the notion that Hsp90 in tumor, as opposed to normal cells, may have an intrinsically greater affinity for GA congeners due to the existence in tumors, but not in normal cells, of a distinct spectrum of Hsp90-associating cochaperone molecule complexes. Thus, a complete understanding of 17-DMAG's intrinsic potency must consider the possibility that tumor-derived Hsp90 complexes will have a greater affinity for 17-DMAG than for 17-AAG. Alternatively, more facile uptake or altered metabolism of 17-DMAG in comparison to 17-AAG might also contribute to its enhanced potency, and both of these issues should be the subject of future investigations with the agent.

The spectrum of antineoplastic activity documented here includes noteworthy activity of 17-DMAG in certain melanoma and pancreatic carcinoma model systems. Banerji et al. [3], in the initial evaluation of an intermittently administered schedule of 17-AAG, have documented protracted disease stabilization of many months to years in certain patients including some with melanoma, accompanied by downregulation of Hsp90 client proteins in both surrogate tissues as well as certain accessible tumor sites. Our experience here as well as that reported previously with 17-AAG [7] points to further interest in evaluating GA congeners in melanoma models. Likewise, GAs are metabolized in the liver and excreted through the bile [10, 33]. The activity of orally administered 17-DMAG in a hepatic and therefore orthotopic model of pancreatic carcinoma metastasis suggests novel approaches to the treatment of established or perhaps even micrometastatic pancreatic carcinoma metastases, or indeed any of a variety of tumors with a propensity to liver metastasis.

Glaze et al. [12] have recently reported the results of toxicity evaluations in rats and dogs. In both species, gastrointestinal and hepatic adverse events were dose limiting, and in dogs, renal toxicity was also apparent. The MTD in rats was 24 mg/m^2 per dose on a daily times five schedule, similar to the 7.5 mg/kg dose in mice which displayed evidence of antitumor activity. Dogs were somewhat more sensitive, with an MTD < 16 mg/m^2 per dose daily times five.

The significance of Hsp90 to a variety of oncogenic pathways including those mediated by various tyrosine kinases and Hsp90 client proteins including certain

Table 2 SC xenograft evaluations (*NE* not evaluable)

Treatment	Dose (mg/kg/dose)	Schedule (day)	Route	Drug-related deaths[a]	Maximal body weight change (%)	Optimal T/C, days (%)	Growth delay (days)[b]
MEXF 276 amelanotic melanoma							
Control		1–3, 8–10,13–17	IV	0/8	+9		
17-DMAG	15	1–3, 8–10, 13–17	IV	1/9	−6	12 (21)**	17
	7.5	1–3, 8–10, 13–17	IV	0/9	−2	31 (21)**	2
	15	1–3,8–10, 13–17 21–24	Oral	1/8	−2	27 (21)**	3
MEXF 989 amelanotic melanoma							
Control		1–5, 8–12,16	IV	1/6	+3		
17-DMAG	15	1–5, 8–12,16	IV	1/7	−27	12 (28)**	55
MEXF514 melanotic melanoma							
Control		1–5, 8–10	IV	0/9	+4		
17-DMAG	15	1–5, 8–10	IV	0/10	−12	70 (21)**	NE
	7.5	1–5, 8–10	IV	0/8	−4	85 (21)	NE
MEXF 462 amelanotic melanoma							
Control		1–3, 8–10	IV	0/8	−16		
17-DMAG	15	1–3, 8–10	IV	1/8	−16	66 (10)	0.4
LXFA 629 adenocarcinoma of the lung							
Control		1–5, 8–12, 16	IV	0/8	+8		
17-DMAG	15	1–5, 8–12, 16	IV	0/9	−13	53 (15)**	6
	7.5	1–5, 8–12, 16	IV	0/7	−2	50 (28)**	6
LXFS 650 small-cell lung carcinoma							
Control		1–4, 9–12, 16	IV	1/7	+12		
17-DMAG	15	1–3, 9–12, 16	IV	0/7	−9	49 (14)**	1.3
	7.5	1–3, 9–12, 16	IV	1/7	−7	46 (28)**	2.8

**$P < 0.05$, Wilcoxon's test
[a]Number of deaths/total number of mice in the group
[b]Growth delay 400% (two control doublings)

nuclear hormone receptors certainly suggests it is an excellent target for deriving novel treatments for neoplastic disease. 17-DMAG represents a potentially valuable means of addressing problems that have arisen with 17-AAG, including the latter's cumbersome formulation for higher doses of administered drug. These formulation concerns limit the possibility of fully investigating many potential clinical uses, since the dosing routes and schedule are limited by the compound's solubility limitations and lack of oral bioavailability. The results reported here offer substantial evidence that 17-DMAG has an excellent potential to expand the horizons of clinical investigations possible with GA congeners, through more practical formulations and additional dosing regimens with the potential for oral routes of administration. Since 17-DMAG has reproducible efficacy in a variety of mouse xenograft models of human cancer the possibility of conveying benefit to patients is supported by the results reported here [16].

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