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ORIGINAL ARTICLE

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A peptidomimetic inhibitor of *ras* functionality markedly suppresses growth of human prostate tumor xenografts in mice. Prospects for long-term clinical utility

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Abstract *Purpose*: These studies sought to evaluate the antitumor properties of an inhibitor of *ras* functionality, L-744,832, which acts at the level of its associated protein farnesyltransferase. *Methods*: Studies were carried out to measure the effects of L-744,832 alone and in combination with paclitaxel (PTXL) against TSU-PR1, DU-145 and PC-3 human prostate tumors xenografted to NCR-nu1 (AT) mice. Tumor-bearing mice were treated on a schedule of daily for 5 days ×2 or 3 with the MTD of L-744,832 and every 3–4 days ×4 with the MTD of PTXL starting 3–5 days after tumor implantation. Tumor volume in millimeters ($4/3\pi r^3$) was measured 3–5 days after cessation of treatment and the increase in tumor volume in treated and control groups compared. Statistical analysis was carried out by the Chi-squared test. *Results*: L-744,832 at its MTD markedly inhibited the growth of all three tumors (T/C for increase in tumor mass varied from 11% to 15% and inhibition of growth had a rapid onset (within 1–2 days) and was independent of *ras* gene status. Estimated tumor doubling times were 8–12-fold greater in treated animals than in control animals. Treatment with L-744,832 for as long as 3 weeks had no untoward effects on the mice as determined by gross examination or necropsy. Administration of L-744,832 with this same dose and schedule potentiated the growth-inhibitory effect of PTXL at its MTD and induced some regression of TSU-PR1 with no obvious deleterious effects on the mice. *Conclusions*: L-744,832 could be safely administered over a protracted period of time to mice at doses which were markedly inhibitory to the growth of three human prostate tumor xenografts and in combination with PTXL was also well tolerated and brought about some regression of the TSU-PR1 tumor. Overall, these results suggest that L-744,832 could be clinically useful for long-term treatment of early-stage prostate cancer in patients and as an adjunct to cytotoxic therapy for late stages of this disease.

Key words Protein farnesylation inhibitor · Human prostate tumors · Efficacy

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Introduction

Overexpression and mutation of *ras* genes are believed [1–5] to result in oncogenic transformation and tumorigenesis, at least in the context of model cellular systems and very likely in human cancer. Alternatively, functional activation of the *ras* gene pathway resulting in transformation could occur [1–5] through autocrine or paracrine mechanisms. Farnesylation of the CAAX box in the COOH terminus of *ras* protein [6, 7] by protein farnesyltransferase is an obligatory step in this transformation. As an approach to more specific therapy of human cancer, inhibitors of *ras* functionality at the level of farnesylation have been designed and evaluated [8–10] as antitumor agents. Among this category of agents, peptidomimetic inhibitors has been shown to have substantial antiproliferative effects against tumor

cells in culture [8, 11] and to block growth of various tumors in animals [12–15] which depend upon expression of *ras* genes. Moreover, these effects are markedly increased [14, 15] against tumors which overexpress these genes. In these cases, tumors with overexpressed activated Ha-*ras* are more sensitive [14] to therapy with these agents than those overexpressing N-*ras* [15].

As most studies with these and other [10] inhibitors have been carried out using transgenic model systems, it was of interest to evaluate their effects against relatively chemoresistant transplantable tumors which express one of the *ras* genes, but are not clonally selected following their oncogenic transformation by these genes. Accordingly, we now report on studies showing that the peptidomimetic inhibitor of protein farnesyltransferase, L-744,832, when administered to mice at a nontoxic dose markedly suppressed the growth of the TSU-PR1, DU-145 and PC-3 human prostate tumors in nude mice. The details of these studies are presented below.

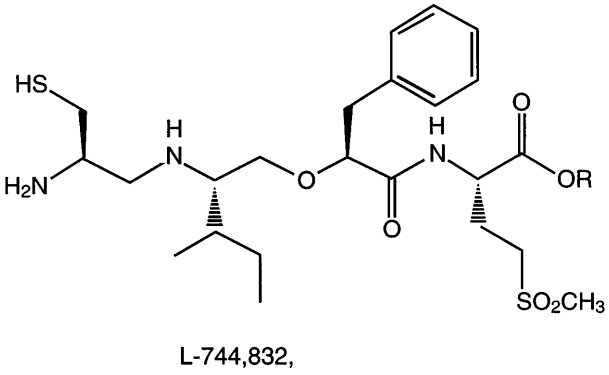
Materials and methods

Cultured TSU-PR1, DU-145 and PC-3 cells were maintained as monolayers in MEM plus 10% fetal calf serum as described previously [16, 17]. For in vivo studies, the TSU-PR1, DU-145 and PC-3 tumors obtained from cell culture were implanted in athymic NCR-nu1 (AT) mice. Cells from culture were centrifuged for 5 min at 1000 *g* and the pellet mixed with an equal volume of Matrigel (Becton-Dickinson, Franklin Lakes, N.J.) prior to subcutaneous implantation into the supraclavical area. After tumor growth, a cell suspension was prepared from the excised implant, centrifuged as above and mixed with an equal volume of Matrigel. Aliquots of this suspension were implanted into a group of mice and 4–7 days later, the mice now bearing tumors 3–4 mm in diameter were randomized between control and treated groups. The maximum tolerated dose (MTD) of L-744,832 used on the prescribed schedule of administration (daily ×5 for 2 or 3 weeks) was determined in preliminary experiments comparing the effects of various doses in tumor-free mice. This dose resulted in 3% weight loss after 2 or 3 weeks of treatment. In other experiments, the mice were also treated with paclitaxel (PTXL) alone or in combination with L-744,832 on a schedule of every 3–4 days ×4.

The average tumor diameter in control (diluent alone) and treated groups at various times after initiation of treatment was obtained from two measurements of the tumor along its shortest and longest axes. The data are expressed as volume (mm^3) of tumor (volume = $4/3\pi r^3$). Statistical analysis was carried out by the Chi-squared test [18]. Other methodological details have been provided in previous reports [16]. L-744,832 (Fig. 1; provided by Merck Research Laboratories, West Point, Pa.) was solubilized [13] in citrate-NaCl buffer (pH 5.4). Working solutions were freshly made or stored for no longer than 1 week at −20 °C. These studies were performed in accordance with "Principles of Laboratory Animal Care" (NIH publication No. 85-23 revised 1985).

Results and discussion

L-744,832 was evaluated in vivo against the TSU-PR1, DU-145 and PC-3 tumors at its MTD of 30 mg/kg given daily for 5 days during two or three successive weeks for a total of 10 or 15 doses. This schedule of administration has been found to be the most appropriate in prior studies by others [13, 14, 15]. The results given in Fig. 2



L-744,832,

Fig. 1 The structure of L-744,832 (2(*s*)-[2(*s*)-[2(*R*)-amino-3-mercapto]-propylamino-3(*s*)-methyl]pentyloxy-3-phenylpropionyl methionine-sulfone isopropyl ester)

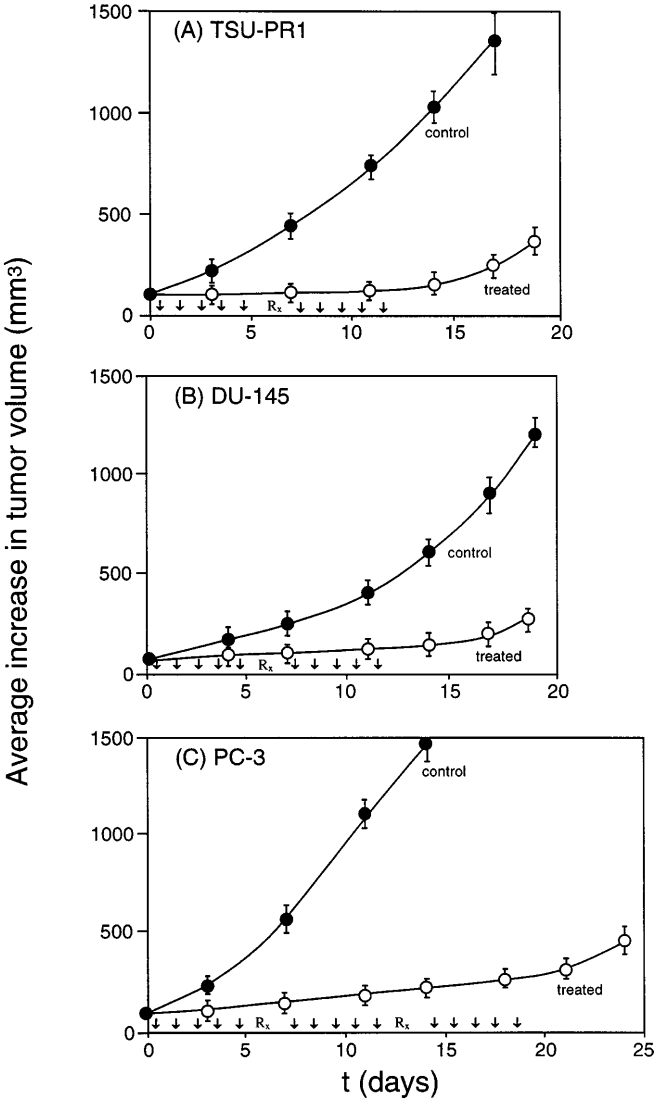


Fig. 2A–C Effect of L-744,832 on the growth of TSU-PR1 (**A**), DU-145 (**B**) and PC-3 (**C**) tumors cells xenografted in AT mice. The data shown are from an average of two experiments varying by less than 15%. Additional details are provided in the text

Table 1 Antitumor effects of L-744,832 against the TSU, DU-145 and PC-3 human prostate tumors (see text for additional details). The data were obtained in two separate experiments with four mice per group. The standard error of the mean (SEM) was determined from the data obtained with the combined total of mice (eight) from each group

Tumor	Group[a]	Average weight change (%)[b]	Average increase in tumor volume[c]		Tumor doubling time (days)[d]	Ras gene status[e]	
			$mm^3 \pm SEM$	%		Type	Relative level
TSU	Control	+1	943 ± 110		2.8	Mu[f]	8–10
	Treatment	−2	102 ± 21	11	33.5		
DU-145	Control	+2	568 ± 97		2.7	wt	< 1
	Treatment	−1	79 ± 18	15	23.0		
PC-3	Control	+1	1390 ± 230		1.5	wt	1
	Treatment	−2	181 ± 34	13	13.0		

[a] 30 mg/kg of L-744,832 daily for 5 days ×2; control animals received diluent alone
[b] Initial weight 28 ± 1 g
[c] Initial tumor volumes (mm^3) were 84 ± 12 (TSU), 67 ± 13 (DU-145) and 62 ± 10 (PC-3). Measurements of tumor volume were made 3 days after the first 2 weeks of treatment with L-744,832
[d] Determined from measurements made of tumor in animals during the initial 7–10 days (control groups) or 14 to 17 days (treated groups) following tumor implantation
[e] As provided in references 10, 11, 12 and 20
[f] Ha*Ras*G12V

and Table 1 show that L-744,832 when administered at this dose and on this schedule markedly inhibited the growth of all three human prostate tumors. The effect on growth occurred with a rapid onset (usually within 1–2 days) which was sustained at the same level during the 2-week (TSU-PR1 and DU-145) or 3-week (PC-3) period of treatment after which time the tumor resumed growth at the same rate as in the controls. The increases in tumor mass observed in the treated groups after 2 weeks of treatment (Table 1) were 89% (TSU-PR1), 85% (DU-145) and 87% (PC-3) less than that determined for the control tumors ($T/C = 11–15\%$, $P \leqslant 0.001$) treated only with diluent. In addition, estimated tumor doubling times were 8–12-fold greater in treated animals than in control animals. At the time of that measurement, the rate of tumor growth in untreated mice only was already beginning to diminish from that seen during the first 5–7 days after transplant. It should be emphasized, that during the course of treatment of the tumor-bearing mice with this agent, no untoward effects were observed either by gross examination or necropsy. However, an extremely modest (1–2%) loss in weight was observed in the group treated with L-744,832 within 1 week of initiation of therapy which did not increase in amount during continuing treatment. Non-tumor-bearing animals were treated at this same dose for as long as 5 weeks (data not shown) with a similar result. It should be noted that weight loss alone was used to establish the MTD for L-744,832 in these experiments since it is the earliest indicator [13–15] of chronic, multiple-dose toxicity in mice. However, it is very likely that optimum dosing with this and related compounds in patients could be determined by means of an end-point related to inhibition of protein farnesyltransferase.

As an adjunct to these studies, we also examined the antitumor effect of L-744,832 in combination with PTXL against the TSU-PR1 tumor. These data are presented in Fig. 3 and show that these agents favorably interacted when administered on their respective schedules during a 2-week period of treatment. While L-744,832 and PTXL when given alone at their MTD brought about an 85% and 92% reduction in growth of TSU-PR1, respectively, in treated mice, combined treatment induced some regression of the tumor. Moreover, the combination of the two agents was well tolerated with treated animals showing a small (2–4%) further increase in weight loss over that seen for each agent alone (data not given) during the period of treatment. No further weight loss or any other manifestation of increased toxicity was observed (data not given) in these animals which were held for an additional 3 weeks after treatment.

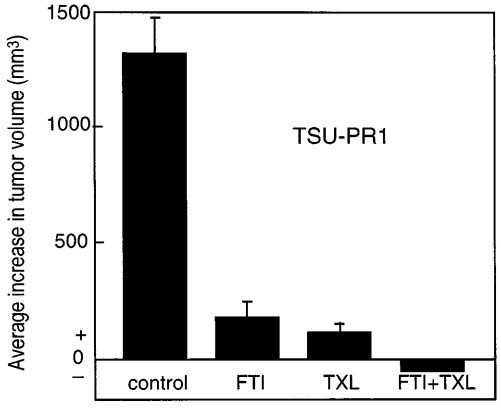


Fig. 3 Effect of L-744,832 with and without PTXL on the growth of the TSU-PR1 tumor xenografted to AT mice. The data shown are from an average of two experiments with the variability shown for each group in the figure. L-744,832 (*FTI*) was administered daily for 5 days during two successive weeks at a dose of 30 mg/kg and paclitaxel (TXL) was administered every 3–4 days ×4 at a dose of 25 mg/kg. Additional details are provided in the text

The data presented here are among the first results from the testing of this class of agent and document appreciable antiproliferative effects against "garden variety" transplantable human prostate tumors in vivo which have not been derived for their overexpression of one of the *ras* gene family members. Some data have been reported very recently [10] showing a similar effect of a nonpeptidomimetic inhibitor of protein farnesyltransferase against a xenograft of the DU-145 tumor.

Our results show that treatment of tumor-bearing AT mice with L-744,832 resulted in essentially an immediate suppression of growth of three human prostate tumors which was substantial yet resulted in no apparent systemic toxicity. They also show that L-744,832 can be safely administered to mice with a cytotoxic agent (PTXL) which resulted in a significant increase in the antitumor response obtained against TSU-PR1. This latter result is interesting in view of evidence presented previously [19] of synergy in the combined effects of L-744,832 and PTXL against the growth of a human breast cancer cell line. While the in vivo data presented here do not allow a similar elucidation of the type of interaction, it is likely that the combined effects of these agents against TSU-PR1 that were observed also reflect a synergistic interaction.

The results with L-744,832 given alone would appear to have important implications for the clinical use of inhibitors of protein farnesyltransferase in the treatment of human prostate cancer. This is especially true for the more indolent early stages of disease where tumor growth rates are usually extremely low. Obtaining the same relative degree of suppression of tumor growth in patients in the manner seen here could have a significant impact on the management of this disease. Although these studies utilized tumors that are not considered sensitive to hormonal treatment, there is no reason to assume that hormone-sensitive tumors would not respond similarly to L-744,832 or related inhibitors of protein farnesyltransferase. In fact, studies in vitro (Sepp-Lorenzino et al., submitted for publication; [20]) have shown that both hormononally responsive and nonresponsive tumors are sensitive to the antiproliferative effect of L-744,839. Therefore, the data presented here would also appear to support the use of these inhibitors as adjuncts to hormonal therapy against early-stage prostrate cancer in patients. Overall, these results bode well for future clinical trials of this class of agent given on a long-term basis to patients with prostate cancer and perhaps other more indolent neoplastic disorders. Our results also suggest that since L-744,832 can be safely and fruitfully administered along with clinically active cytotoxic agents, inhibitors of protein farnesyltransferase may have a meaningful role in the treatment of more aggressive late-stage disease.

It may also be inferred from these studies that overexpression, mutation or functional activation of *ras* may not be a necessary requirement for antitumor response to L-744,832 and related inhibitors of *ras* functionality, at least in this experimental setting. This conclusion is consistent with the results of earlier biochemical studies (Sepp-Lorenzino et al., submitted for publication; [20]) and is derived here from the fact that the three prostate tumors studied, all of which have an activated *ras* pathway [11], are very different (Table 1) with respect to the status of this gene, yet all responded similarly to L-744,832. The DU-145 and PC-3 tumors express low but different levels of Ha-*ras*, respectively, while the TSU-PR1 tumor overexpresses mutated Ha-*ras*. A similar lack of correlation between *ras* gene status and cytotoxicity of L-744,832 against these same tumors has been also observed [20] in cell culture studies. The basis for this lack of correlation between *ras* status and response to this agent in these tumors is unclear, given that nothing is known as to the cellular pharmacokinetic behavior and metabolic disposition of L-794,832 in these tumors. Both factors could have a significant impact on responsiveness to this agent. In addition, there may be other molecular differences among these tumors, including those related to downstream elements in the *ras* pathway, which also influence [19, 20] the response of these tumors to this agent. Additional work will hopefully shed light on these issues. However, the fact remains that L-744,832 exhibited highly interesting effects against these three human tumor xenografts in the present studies.

References

1. DeFeo D, Gonda MA, Young HA, Chang EH, Lowry DR (1981) Analysis of two divergent rat genomic clones homologous to the transforming gene of Harvey murine sarcoma virus. Proc Natl Acad Sci USA 78: 3328–3332
2. Shah C, Weinberg RA (1982) Isolation of a transforming sequence from a human bladder cancer carcinoma cell line. Cell 29: 161–169
3. McKay IA, Marshall C, Cales C, Hall A (1986) Transformation and stimulation of DNA synthesis in NIH3T3 cells are a titratable function of normal $p21^{N\text{-}ras}$ expression. EMBO J 5: 2617–2621
4. Sinn E, Muller W, Pattengale P, Tepler I, Wallace R, Leder P (1987) Coexpression of MMTV/v-H-*ras* and MMTV/c-myc genes in transgenic mice: synergistic action of oncogenes in vivo. Cell 49: 465–475
5. Mangues R, Seidman I, Gordon JW, Pelicer A (1992) Overexpression of the V-*ras* proto-oncogene, not somatic mutational activation, associated with malignant tumors in transgenic mice. Oncogene 7: 2073–2076
6. Schafer WR, Kim R, Sterne RE, Thorner J, Kim SH, Rine J (1989) Genetic and pharmacological suppression of oncogenic mutations in *ras* genes of yeast and humans. Science 254: 379–385
7. Hancock JF, Magee AI, Childs JE, Marshall CJ (1989) All *ras* proteins are polyisoprenylated but only some are palmitoylated. Cell 57: 1167–1177
8. Kohl NE, Mosser SD, deSolms SJ, Grulsani EA, Pompliano DL, Graham SL, Smith RL, Scolnick EM, Oliff A, Gibbs JB (1993) Selective inhibition of *ras*-dependent transformation by a farnesyltransferase inhibitor. Science 260: 1934–1937
9. James GL, Goldstein JL, Brown MS, Rawson TE, Somers TC, McDowell RS, Crowley CW, Lucas BK, Levinson AD, Masters JC (1993) Benzodiazepine peptidomimetics: potent inhibitors of *ras* farnesylation in animal cells. Science 260: 1937–1942
10. Liu M, Bryant MS, Chen J, Lee S, Yaremko B, Lipari P, Malkowski M, Ferrari E, Nielsen L, Prioli N, Dell J, Sinha D, Syed J, Walter KA, Nomeir AA, Lin C-C, Wang L, Taveras AG, Doll RJ, Njoroge FG, Mallams AK, Remiszewski S, Cation JJ, Girijavallabhan VM, Kirschmeier P, Bishop WR (1998) Antitumor activity of SCH 66336, an orally bioavailable tricyclic inhibitor of farnesyl protein transferase, in human xenograft models and wap-*ras* transgenic mice. Cancer Res 58: 4947–4956
11. Sepp-Lorenzino L, Ma Z, Rands E, Kohl NE, Gibbs JB, Oliff A, Rosen N (1995) A peptidomimetic inhibitor of farnesyl: protein transferase blocks the anchorage dependent and inde-

pendent growth of human tumor cells lines. Cancer Res 55: 5302–5309
12. Carter BS, Epstein JI, Isaacs WB (1990) *ras* gene mutations in human prostate cancer. Cancer Res 50: 6830–6832
13. Kohl NE, Wilson FR, Mosser SD, Guiliani EA, DeSolma SJ, Conner MW, Anthony NJ, Holtz WJ, Gomez RP, Lee TJ, Smith RL, Graham SL, Hartman GD, Gibbs JB, Oliff A (1994) Protein farnesyltransferase inhibitors block the growth of *ras*-dependent tumors in nude mice. Proc Natl Acad Sci USA 91: 9141–9145
14. Kohl NE, Omer CA, Conner MW, Anthony NJ, Davide JP, deSolma SJ, Guiliani EA, Gomez RP, Graham SL, Hamilton K, Handt LK, Hartman GD, Koblan KS, Kral AM, Miller PJ, Mosses SD, O'Neill TJ, Randa E, Schaner MD, Gibbs JB, Oliff A (1995) Inhibition of farnesyltransferase induces regression of mammary and salivary carcinomas in *ras* transgenic mice. Nat Med 1: 792–797
15. Mangues R, Coral T, Kohl NE, Symmans WF, Lu S, Malumbres M, Gibbs JB, Oliff A, Pellicer A (1998) Antitumor effects of a farnesyl protein transferase inhibitor in mammary and lymphoid tumors over expressing N-*ras* in transgenic mice. Cancer Res 58: 1253–1259
16. Chou T-C, Otter GM, Sirotnak FM (1996) Schedule-dependent synergism of Taxol and Taxotere with edatrexate against human breast cancer cells in vitro. Cancer Chemother Pharmacol 37: 222–228
17. Sirotnak FM, DeGraw JI, Colwell WT, Piper JR (1998) A new analogue of 10-deazaaminopterin with markedly enhanced curative effects against human tumor xenografts in mice. Cancer Chemother Pharmacol 42: 313–318
18. Zar JH, Biostatistical analysis, 2nd edn. Prentice Hall, Englewood Cliffs, pp 145–146
19. Moasser MM, Sepp-Lorenzino L, Kohl NE, Oliff A, Balog A, Su Dai-Shi, Danishesfesky SJ, Rosen N (1998) Farnesyl transferase inhibitors cause enhanced sensitivity to Taxol and epithilones. Proc Natl Acad Sci USA 95: 1369–1374
20. Sepp-Lorenzino L, Rosen N (1998) A farnesyl-protein transferase inhibitor induces p21 expression and G_1 block in p53 wild-type tumor cells. J Biol Chem 273: 20243–20251