Thalidomide Radiosensitization of Normal Murine Hematopoietic but Not Squamous Cell Carcinoma or Multiple Myeloma Tumor Cell Lines

MICHAEL W. EPPERLY, EMILY E. GREENBERGER, DARCY FRANICOLA,
SAMUEL JACOBS and JOEL S. GREENBERGER

*Departments of Radiation Oncology and Medicine, University of Pittsburgh Cancer Institute
and University of Pittsburgh School of Medicine, Pittsburgh, PA, U.S.A.*

Abstract. *Background: Thalidomide (TL), due to its anti-angiogenic effects, has been postulated to be a potential radiosensitizer of multiple myeloma and squamous tumors in vivo. Materials and Methods: To determine whether TL was a radiosensitizer, 32D cl 3 cells (hematopoietic progenitor) as well as SCC-VII (squamous cell carcinoma), OPM1 or OPM2 (multiple myeloma) tumor cells were irradiated to doses ranging from 0 to 8 Gy and then plated in 0, 50 or 150 μM TL in each of three protocols: i) 1 hour before irradiation; ii) 1 hour before irradiation and also in medium following irradiation; or iii) placed in TL containing medium following irradiation. Results: Using 150 μM TL (which did not stimulate cell growth) the 32D cl 3 cells had increased radiation sensitivity compared to the control irradiated cells. In contrast, the SCC-VII, OPM1 or OPM2 cells showed no detectable radiosensitization when incubated in TL before, during or after irradiation compared to the control irradiated cells. Conclusion: These results demonstrated that TL may be a selective radiosensitizer.*

The anti-angiogenic properties of thalidomide (TL) suggested that this drug must be a valuable addition to combined modality protocols for the treatment of malignancies in which neovascularization is a component of tumor drug resistance and/or recurrence (1, 2). In particular, multiple myeloma has been demonstrated to induce neo-angiogenesis in the bone marrow and also production of angiogenic cytokines by bone marrow stromal cells in the hematopoietic microenvironment of the marrow

(1, 2). Furthermore, the anti-angiogenic properties of TL have suggested its potential use in combined modality protocols for the treatment of other hematological malignancies (2-3), or squamous cell tumors including non-small cell lung carcinoma, and tumors of the head and neck region (3-5).

The complex interactions between tumor cells, bone marrow stromal cells, reticular adventitial cells (endothelial cells), and other hematopoietic cells in the micro-environment of the bone marrow make it difficult to determine the specific effects of a new chemotherapeutic or biological response modifying agent on tumor cells independent of tumor cell interaction with one or many other cell types (6-10). To determine whether TL had a direct effect on the radiosensitivity of tumor cells, assays were carried out for clonogenic survival of multiple myeloma or squamous cell carcinoma cell lines derived from murine models of each malignancy. The results were compared with those for the Interleukin-3 (IL-3)-dependent hematopoietic progenitor cell line 32D cl 3 (11), which forms colonies in semi-solid medium in the presence of hematopoietic growth factors (12). The results demonstrated TL-mediated radiosensitization of the non-malignant hematopoietic progenitor cells compared to either squamous cell carcinoma or multiple myeloma tumor cell lines.

Materials and Methods

Cell lines and cell culture. The SCC-VII (13) murine squamous cell carcinoma cell line has been described and the murine multiple myeloma cell lines OPM1 and OPM2 (a generous gift of Dr. G. David Roodman, MD, Department of Medicine, UPCI, USA) (14) were grown in DMEM medium containing 10% fetal calf serum (FCS). The 32D cl 3 cells (11) were grown in RMPI-1640 medium supplemented with 15% WEHI-3 cell-conditioned medium as a source of IL-3 and 15% FCS, penicillin and streptomycin, according to published procedures. The cells were passaged weekly at high density (15).

Correspondence to: Joel S. Greenberger, MD, Professor and Chairman, Department of Radiation Oncology, University of Pittsburgh Medical School, 200 Lothrop Street, Pittsburgh, PA 15213, U.S.A.

Radiation survival curves. The cells were incubated in TL at concentrations of 50 or 150 μM either 1 hour before irradiation, after irradiation by placing the cells in methylcellulose or DMEM medium containing 50 or 150 μM TL, or both before and after irradiation. The cells were irradiated using a Cesium 35 Gamma irradiator to doses ranging from 0 to 8 Gy, according to published methods (16, 17). The 32D cl 3 cells or mouse bone marrow cells were plated in methylcellulose containing 15% WEHI-3 cell line (IL-3)-conditioned medium and 10 % FCS and incubated at 37˚C and 5% CO_2 in a humified incubator for 7 days, at which time colonies of greater than 50 cells were counted (15). Following irradiation, the SCC-VII, OPM1 or OPM2 cells were plated in four-well tissue culture plates according to published methods (18), incubated for 5 days at 37˚C and 5% CO_2 in a humified incubator, at which time the cells were stained with crystal violet and colonies of more than 50 cells counted. The data were analyzed by linear quadratic and single-hit, multi-target models.

Effects of TL on cell growth. 32D cl 3 cells were plated at 500 cells per dish in methylcellulose containing 15% WEHI-3-conditioned medium, 10% FCS serum and concentrations of TL ranging from 0 to 200 μM. Seven days later, colonies of more than 50 cells were counted. SCC-VII cells were plated at 500 cells per well in four-well plates and grown in DMEM medium containing 10% FCS and concentrations ranging from 0 to 200 μM. The cells were stained with crystal violet 5 days later and colonies of more than 50 cells were counted.

Effects of TL on IL-3 dependency of 32D cl 3 cells. 32D cl 3 cells were plated in methylcellulose containing 50 or 150 μM TL plus each of several 10-fold dilutions of WEHI-3-conditioned medium as a source of IL-3 ranging from 0 to 15%. Seven days later colonies of more than 50 cells were counted.

Statistical evaluation. The Student's *t*-test and evaluation of D_0, ñ, and alpha β components of the linear quadratic radiation survival curves were carried out according to published methods (19).

Results

Radiosensitization of 32D cl 3 hematopoietic progenitor cells by TL. Radiation survival curves of 32D cl 3 cells were first carried out according to the methods section. The results with 50 μM and 150 μM TL are shown in Figure 1 and Figure 2, respectively. In 150 μM TL (Figure 2), but not 50 μM (Figure 1), there was radiosensitization of 32D cl 3 cells as indicated by a decrease in the D_0 relative to control irradiated cells (Table I).

The effect of TL on colony formation by unirradiated 32D cl 3 cells grown in IL-3-conditioned medium was measured next. As shown in Figure 3, there was an increase in colony formation induced by doses as low as 25 μM TL, which increased the growth of 32D cl 3 cells over the dose range of 25 μM to 200 μM (50-150 μM TL) in constant 10% IL-3-containing medium. Doses of 250 μM TL caused clear toxicity (data not shown).

It was next determined whether therapeutic-range doses of TL would induce growth of IL-3-dependent 32D cl 3 cells in lower concentrations of IL-3. This experiment was carried out to determine whether, in cases of depletion of IL-3, TL would stimulate growth or whether, alternatively, a paracrine or additive effect of TL would be seen to induce colony formation at lower concentrations of IL-3. A dose response curve of cells grown in TL at a constant 50 μM (Figure 4A) or 150 μM (Figure 4B) in the presence of increasing concentrations of IL-3 (over 1% to 15%) showed modest stimulation of colony growth at the low but not the high dose. There was no significant stimulatory effect of TL alone (no IL-3) over the dose range of 50-150 μM.

Lack of detectable TL-mediated radiosensitization of SCC-VII squamous cell carcinoma cells. The murine floor of the mouth squamous cell carcinoma cell line SCC-VII was grown *in vitro* according to published methods (13). Cells were irradiated to doses between 1 and 10 Cy and then plated in plastic Petri dishes for scoring adherent cell colonies of over 50 cells per colony on day 5. The results are shown in Figure 5. The SCC-VII cells showed no significant relative radiosensitization or radioresistance when grown in the clinically-relevant dose of 150 μM TL. There was also no effect with 50 μM TL (data not shown). Growth assays of the SCC-VII cells in TL alone over the range 50 μM to 200 μM were next carried out. TL in the absence of irradiation induced no detectably significant alteration in cell colony formation by SCC-VII cells (Figure 6). Thus, in clear contrast to units with 32D cl 3 cells, there was no detectable effect of TL with respect to the radiation survival or growth stimulation of SCC-VII cells.

Lack of detectable TL-mediated radiosensitization of murine multiple myeloma cells. The cell lines OPM1 and OPM2 were grown in semi-solid medium and irradiation survival curves carried out as described in Materials and Methods. The results are shown in Figure 7. Both murine multiple myeloma cell lines demonstrated D_0 with more similarity to the 32D cl 3 cells than to the squamous cell carcinoma cell line SCC-VII, consistent with the hematopoietic origin of multiple myeloma tumors. In the presence of 50 μM TL there was no effect on radiosensitivity (not shown). At the therapeutic dose of 150 μM TL (Figure 7), there was no detectable radiosensitization or induction of radioresistance in the multiple myeloma cells treated with TL, either before or after irradiation.

Discussion

Combined modality protocols, utilizing chemotherapy and radiation, have gained increasing acceptance in the management of patients with hematological malignancies as
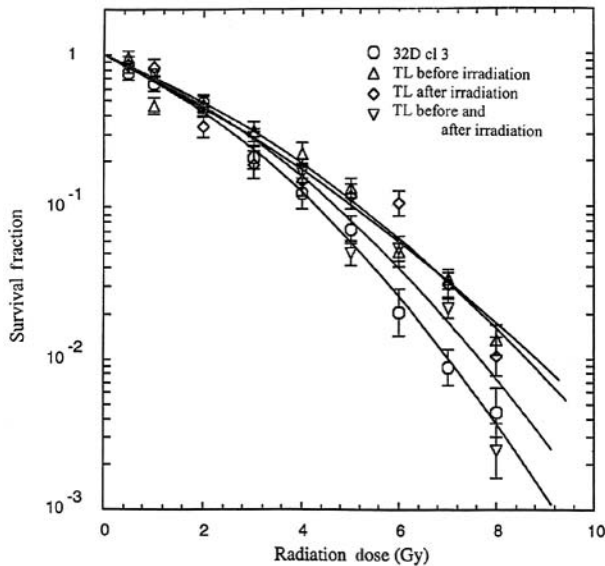
Figure 1. *Incubation of 32D cl 3 cells in 50 μM TL does not alter radiosensitivity. 32D cl 3 cells were incubated in the presence of 50 μM TL 1 hour before irradiation, following irradiation, or both before and after irradiation. The data were analyzed by linear quadratic and single-hit, multi-target models. There was no significant difference in the radiosensitivity measured by D_0 or ñ (Table I).*
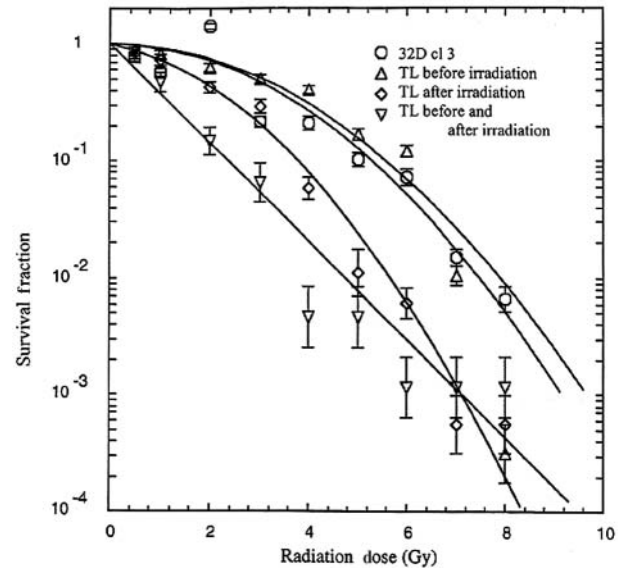
Figure 2. *Radiosensitivity increased in 32D cl 3 cells by 150 μM TL added after irradiation, or present both before and after irradiation. Cells were incubated in 150 μM TL and irradiated as described in the legend to Figure 1. Cells incubated in TL either after, or both before and after, irradiation had increased radiosensitivity (decreased D_0 and shoulder on the irradiation survival curve ñ compared to unirradiated cells or cells incubated in TL only before irradiation) (Table I).*

Table I. *Lack of detectable TL effect on radiosensitivity of squamous cell carcinoma or multiple myeloma tumor cell lines. Cells from 32D cl 3, SCC-VII, OPM1 or OPM2 were incubated in 50 or 150 μM TL 1 hour before irradiation, following irradiation, or both before and after irradiation. Control cells were not exposed to TL. TL had no significant effect on the baseline radiosensitivity of the tumor cell lines.*

Cell line		D_0 (Gy)				ñ			
	TL (μM)	Control	Before	After	Before±After	Control	Before	After	Before±After
32D cl 3	50	1.19±0.06	2.25±0.54	1.36±0.27	1.30±0.11	3.6±0.6	1.8±0.6	1.1±0.1	2.5±0.8
32D cl 3	150	1.23±0.06	1.49±0.48	0.93+ 0.06*	0.89±0.08**	7.3±6.2	16.4±13.8	6.9±4.2	1.8±0.12
SCC-VII	150	1.72±0.24	1.90±0.33	1.63±0.28	1.80±0.40	12.5±6.2	14.6±5.7	14.3±6.2	25.2±16.1
OPM1	150	1.10±0.07	1.11±0.19	0.86±0.04	1.13±0.16	5.3±3.4	11.0±10.0	46.9±35.6	4.1±2.4
OPM2	150	1.25±0.21	1.26±0.29	1.19±0.15	1.25±0.27	3.4±1.9	43.7±41.3	7.7±1.2	7.8±5.7

(*) Significant increase in sensitivity compared to control p=0.0177, (**) p=0.0098. These repeat experiments are not the same as those in the figures.

←
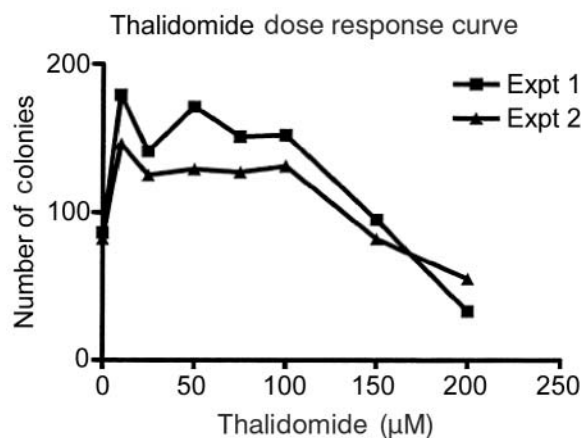
Figure 3. *Increased colony formation by 32D cl 3 cells incubated with low concentrations of TL. Cells from 32D cl 3 were incubated in 10% IL-3 methylcellulose-containing medium (26) in concentrations of TL ranging from 0 to 200 μM. Colonies of more than 50 cells were counted on day 7. At TL concentrations ranging from 10 to 100 μM, there was increased colony formation of 32D cl 3 cells in two separate experiments, compared to results with 32D cl 3 cells plated in IL-3-containing methylcellulose in the absence of TL. At concentrations of greater than 150 μM, there was decreased colony formation indicating that concentrations of more than 100 μM were toxic to the cells.*
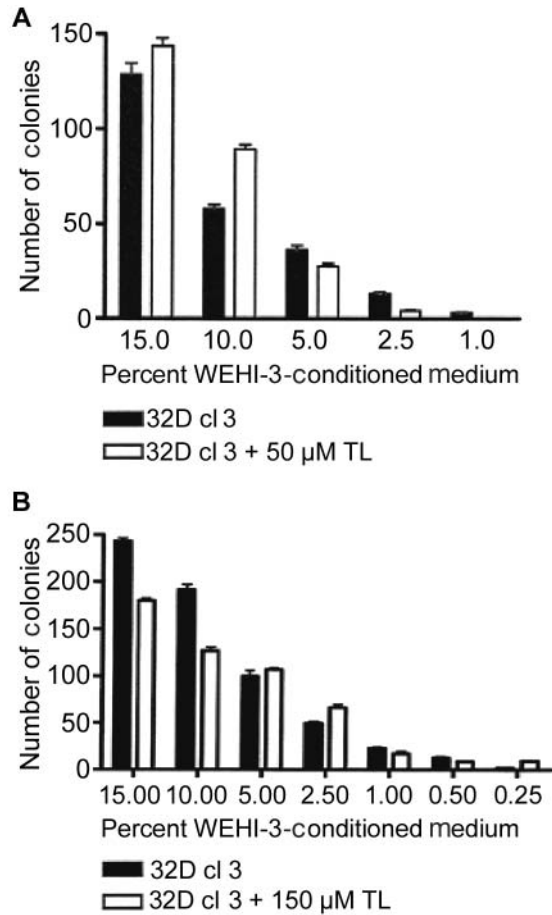
Figure 4. *TL effect on IL-3 dependency of 32D cl 3 colony growth. Cells from 32D cl 3 were incubated in methylcellulose-containing medium with 50 μM (A) or 150 μM (B) TL added and each of several concentrations of WEHI-3-conditioned medium as a source of IL-3. Colonies of more than 50 cells were counted on day 7. Low concentrations of WEHI-3-conditioned medium resulted in decreased colony formation. The presence of TL had no significant effect on stimulation of colony formation in the absence of or in low concentrations of IL-3.*

Figure 5. *TL did not sensitize SCC-VII tumor cells to irradiation. SCC-VII cells were incubated in 150 μM TL before, after, or both before and after irradiation. The cells were plated in four-well tissue culture plates, stained 5 days later with crystal violet and colonies of more than 50 cells were counted. The data were analyzed using linear quadratic and single-hit, multi-target models. There was no significant change in the radiosensitivity of SCC-VII cells when incubated in 150 μM TL (Table I).*

Figure 6. *TL had no detectable significant effect on SCC-VII tumor cell colony formation in vitro. SCC-VII cells were plated in four-well plates and TL at concentrations ranging from 0 to 200 μM, as described in Materials and Methods. The cells were stained 5 days later with crystal violet and colonies of more than 50 cells were counted. There was no significant change in colony formation induced by any of the TL concentrations.*

well as epithelial cancers (1-5). In particular, the management of multiple myeloma has been shown to be significantly improved with respect to decreased toxicity and improved quality of life when patients receive chemotherapy supplemented with biological response modifiers (1). Biological response modifiers are those agents demonstrated to have no specific cytotoxic effect against tumor cells, but to have significant antitumor activity based on indirect effects through either modulation of tumor induction of its blood supply, inhibition of tumor cell interaction/adhesion with cells in the hematopoietic microenvironment, or specific stimulation of T-cells, natural killer cells, or macrophages which provide improved tumor control. One recently evaluated biological response modifier has been TL.
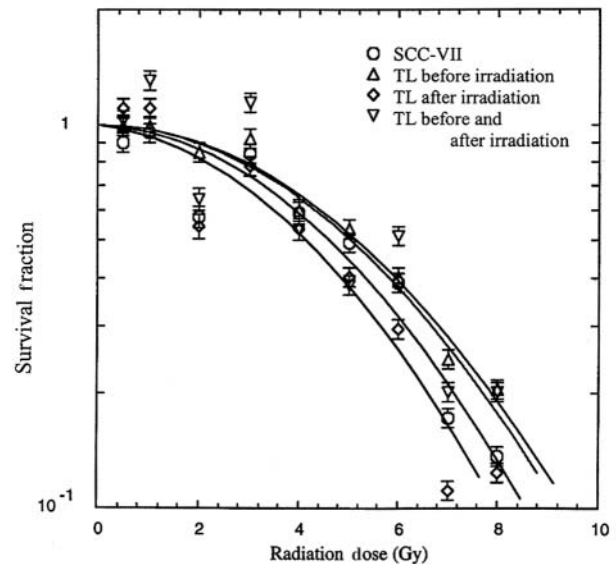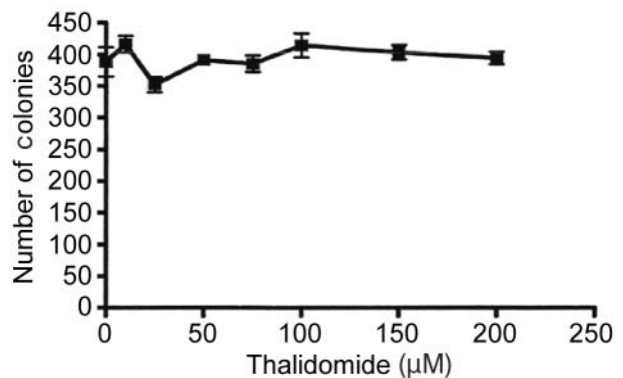
TL was originally used in the 1950s as an antidepressant/sedative drug (2) but, due to its teratogenic properties in developing human embryos, was taken off the market and not utilized for decades (2). Recognition of the anti-angiogenic properties of TL, possibly explaining its
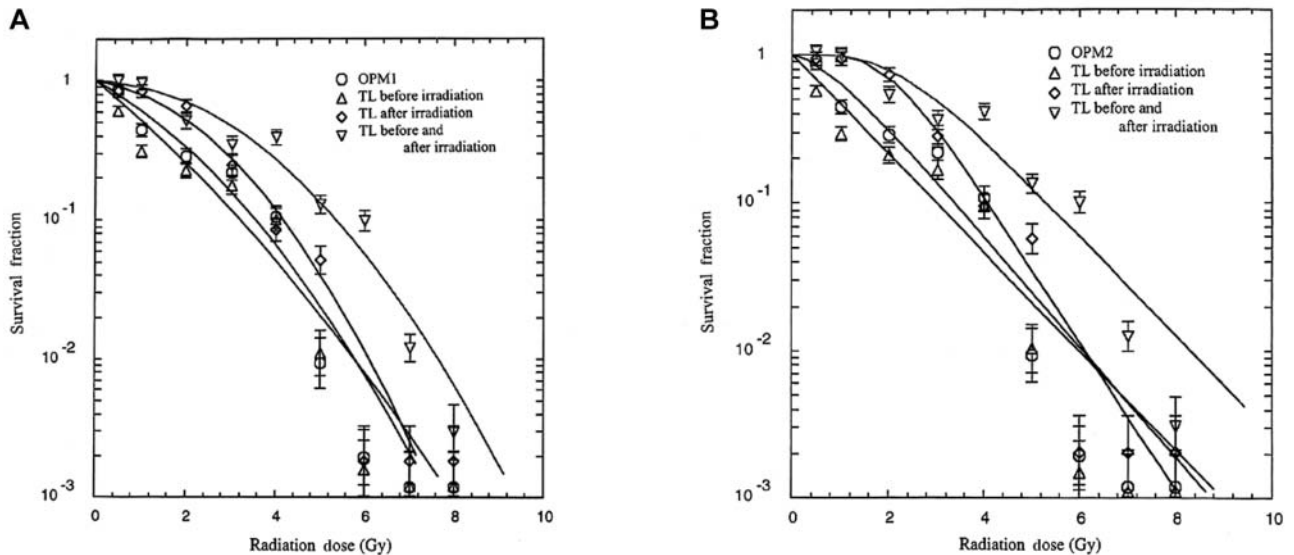
Figure 7. *Radiosensitivity of OPM cells was not altered by TL. Mouse multiple myeloma cells from OPM1 (A) or OPM2 (B) were incubated in 150 μM TL before, after, or both before and after irradiation doses ranging from 0 to 8 Gy, plated in four-well tissue culture plates, stained 5 days later with crystal violet and colonies of more than 50 cells counted. The data were analyzed by single-hit, multi-target and linear quadratic models. There was no significant change in the radiosensitivity of cells incubated in TL, compared to cells grown in the absence of TL (Table I).*

teratogenic effects, suggested that the drug might be of benefit in the management of patients with malignancies. Initial clinical trials of TL in combination with chemotherapeutic drugs showed success in improving tumor response in patients with multiple myeloma (1, 2). Multiple myeloma was chosen as a tumor in which to test TL because of recent evidence indicating that multiple myeloma cells induce a significant angiogenic response in the bone marrow (6). Such angiogenesis has also been found with human acute myelogenous leukemia, as well as solid tumors (2). Another significant tool in the management of drug-resistant multiple myeloma has been total body irradiation followed by bone marrow transplantation (2). Since total body irradiation is utilized not only to clear spaces in the bone marrow for engrafting hematopoietic cells, but also to provide for cytoreduction of residual multiple myeloma cells, we sought to determine whether TL, commonly used as a tumor agent prior to total body irradiation, could be acting as a radiosensitizer or radioprotector for surviving multiple myeloma cells.

We first tested the effects of TL on the radiation survival curve of the murine hematopoietic progenitor cell line 32D cl 3. There was significant radiosensitization of the 32D cl 3 cells in the presence of clinically-relevant concentrations of 50-150 μM TL. In contrast, there was no detectable radiosensitization of SCC-VII or each of the two murine multiple myeloma cell lines.

The results indicate that there may be no significant therapeutic effect of TL specific for tumor, independent of

its known anti-angiogenic- and hematopoietic micro-environment-modifying capacities. Due to the possible radiosensitizing effects of this drug for normal hematopoietic cells perhaps being related to its growth modifying effects, the presented results support added caution in the use of irradiation in conjunction with TL. Other potential therapeutic drugs are being evaluated for use in the treatment of multiple myeloma patients (1, 10, 20-29, 30-32) and patients with other tumors (33, 34). A significant problem in bone marrow transplantation is recovery of hematopoietic cell proliferation differentiation to neutrophils, erythrocytes and lymphocytes, as well as megakaryocytic and platelets in the recovering marrow transplant recipient. TL usage in conjunction with total body irradiation may provide an unwanted radiosensitizing effect with greater kill of normal hematopoietic progenitors. Therefore, design of protocols for utilization of TL in combination with other particularly radiomimetic chemotherapeutic agents should take into account the potential radiosensitization properties of this valuable antitumor agent.

Acknowledgements

References

1 Badros AZ, Goloubeva O, Rapoport AP, Ratterree B, Gahres N, Meisenberg B, Takebe N, Heyman M, Zweibel J, Streicher H, Gocke CD, Tomic D, Flaws JA, Zhang B and Fenton RG: Phase II study of G3139, a Bcl-2 antisense oligonucleotide, in combination with dexamethasone and thalidomide in relapsed multiple myeloma patients. J Clin Oncol 23: 4089-4099, 2005.

2 Ribatti D and Vacca A: Therapeutic renaissance of thalidomide in the treatment of haematological malignancies. Leukemia *19*: 1525-1531, 2005.

3 Chanan-Khan A, Miller KC, Takeshita K, Koryzna A, Donohue K, Bernstein ZP, Mohr A, Klippenstein D, Wallace P, Zeldis JB, Berger C and Czuczman MS: Results of a phase I clinical trial of thalidomide in combination with fludarabine as initial therapy for patients with treatment-requiring chronic lymphocytic leukemia (CLL). Blood *106(10)*: 3348-3355, 2005.

4 Kaufmann H, Raderer M, Wohrer S, Puspok A, Bankier A, Zielinski C, Chott A and Drach J: Antitumor activity of rituximab plus thalidomide in patients with relapsed/ refractory mantle cell lymphoma. Blood *104*: 2259-2271, 2004.

5 Li X, Liu X, Wang J, Wang Z, Jiang W, Reed E, Zhang Y, Liu Y and Li QQ: Thalidomide down-regulates the expression of VEGF and bFGF in cisplatin-resistant human lung carcinoma cells. Anticancer Res *23*: 2481-2488, 2003.

6 Vacca A, Scavelli C, Montefusco V, DiPietro G, Neri A, Mattioli M, Bicciato S, Nico B, Ribatti D, Dammacco F and Corradini P: Thalidomide downregulates angiogenic genes in bone marrow endothelial cells of patients with active multiple myeloma. J Clin Oncol *23*: 5334-5346, 2005.

7 Koh K-R, Janz M, Mapara MY, Lemke B, Stirling D, Dorken B, Zenke M and Lentzsch S: Immunomodulatory derivative of thalidomide (IMiD CC-4047) induces a shift in lineage commitment by suppressing erythropoiesis and promoting myelopoiesis. Blood *105(10)*: 3833-3841, 2005.

8 Ehrlich LA, Chung HY, Ghobrial I, Choi SJ, Morandi F, Colla S, Rizzoli V, Roodman GD and Giuliani N: IL-3 is a potential inhibitor of osteoblast differentiation in multiple myeloma. Blood *106(4)*: 1407-1414, 2005.

9 Hodge DR, Xiao W, Peng B, Cherry JC, Munroe DJ and Farrar WL: Enforced expression of superoxide dismutase 2/manganese superoxide dismutase disrupts autocrine Interleukin-6 stimulation in human multiple myeloma cells and enhances dexamethasone-induced apoptosis. Cancer Res *65*: 6255-6262, 2005.

10 Vincent L, Jin DK, Karajannis MA, Shido K, Hooper AT, Rashbaum WK, Pytowski B, Wu Y, Hicklin DJ, Zhu Z, Bohlen P, Niesvizky R and Rafii S: Fetal stromal-dependent paracrine and intracrine vascular endothelial growth factor-A/vascular endothelial growth factor receptor-1 signaling promotes proliferation and motility of human primary myeloma cells. Cancer Res *65(8)*: 3185-3192, 2005.

11 Greenberger JS, Sakakeeny MA, Humphries KC, Eaves CG and Eckner RJ: Demonstration of permanent factor-dependent multipotential (erythroid/neutrophil/basophil) hematopoietic progenitor cell lines. PNAS USA *80*: 2931-2935, 1983.

12 FitzGerald TJ, Rothstein L, Kase K and Greenberger JS: Radiosensitivity of human bone marrow granulocyte-macrophage progenitor cells: effect of dose rate on purified target cell populations. Radiat Res *107*: 205-215, 1986.

13 Guo H, Seixas-Silva JA, Epperly MW, Gretton JE, Shin DM, Bar-Sagi D, Archer H and Greenberger JS: Prevention of radiation-induced oral cavity mucositis by plasmid/liposome delivery of the human manganese superoxide dismutase (SOD2) transgene. Radiat Res *159(3)*: 361-370, 2003.

14 Katagiri S, Yonezawa T, Kuyama J, Kanayama Y, Nishida K, Abe T, Tamaki T, Ohnishi M and Tarui S: Two distinct human myeloma cell lines originating from one patient with myeloma. Int J Cancer *36(2)*: 241-246, 1985.

15 Epperly MW, Gretton JE, Bernarding M, Nie S, Rasul B and Greenberger JS: Mitochondrial localization of copper/zinc superoxide dismutase (Cu/ZnSOD) confers radioprotective functions *in vitro* and *in vivo*. Radiat Res *160*: 568-578, 2003.

16 FitzGerald TJ, Henault S, Sakakeeny MA, Santucci MA, Pierce JH, Anklesaria P, Kase K, Lovington K and Greenberger JS: Resistance of hematopoietic stem cells to X-irradiation at clinical low-dose-rate is induced by several classes of oncogenes. Radiat Res *122*: 44-53, 1990.

17 Epperly MW, Bray JA, Esocobar P, Bigbee WL, Watkins S and Greenberger JS: Overexpression of the human MnSOD transgene in subclones of murine hematopoietic progenitor cell line 32D cl 3 decreases irradiation-induced apoptosis but does not alter G2/M or G1/S phase cell cycle arrest. Radiat Oncol Invest Clin Basic Res *7*: 331-342, 1999.

18 Epperly MW, Bray JA, Carlos TM, Prochownik E and Greenberger JS: Biology of marrow stromal cell lines derived from long-term bone marrow cultures of Trp53-deficient mice. Radiat Res *152*: 29-40, 1999.

19 FitzGerald TJ, Kase K, Daugherty C, Rothstein L and Greenberger JS: Effect of X-irradiation dose rate on the clonogenic survival of human and experimental animal hematopoietic tumor cell lines: evidence for heterogeneity. IJROBP *12*: 69-73, 1986.

20 Ishitsuka K, Hideshima T, Hamasaki M, Raje N, Kumar S, Podar K, Le Gouill S, Shiraishi N, Yasui H, Roccaro AM, Tai Y-Z, Chauhan D, Fram R, Tamura K, Jain J and Anderson KC: Novel inosine monophosphate dehydrogenase inhibitor VX-944 induces apoptosis in multiple myeloma cells primarily *via* caspase-independent AIF/Endo G pathway. Oncogene *24*: 5888-5896, 2005.

21 Chauhan D, Li G, Podar K, Hideshima T, Neri P, He D, Mitsiades N, Richardson P, Chang Y, Schindler J, Carver B and Anderson KC: A novel carbohydrate-based therapeutic GCS-100 overcomes bortezomib resistance and enhances dexamethasone-induced apoptosis in multiple myeloma cells. Cancer Res *65(18)*: 8350-8356, 2005.

22 Goel A, Dispenzieri A, Greipp PR, Witzig TE, Mesa RA and Russell SJ: PS-341-mediated selective targeting of multiple myeloma cells by synergistic increase in ionizing radiation-induced apoptosis. Exp Hematol *33*: 784-795, 2005.

23 Tovari J, Gilly R, Raso E, Paku S, Bereczky B, Varga N, Vago A and Timar J: Recombinant human erythropoietin α targets intratumoral blood vessels, improving chemotherapy in human xenograft models. Cancer Res *65(16)*: 7186-7193, 2005.

24 Wu K-D, Cho YS, Katz J, Ponomarev V, Chen-Kiang S, Danishefsky SJ and Moore MAS: Investigation of antitumor effects of synthetic epothilone analogs in human myeloma models *in vitro* and *in vivo*. PNAS *102(30)*: 10640-10645, 2005.

25 Tassone P, Neri P, Carrasco DR, Burger R, Goldmacher VS, Fram R, Munshi V, Shammas MA, Catley L, Jacob GS, Venuta S, Anderson KC and Munshi NC: A clinically relevant SCID-hu *in vivo* model of human multiple myeloma. Blood *106(2)*: 713-720, 2005.

26 Dong D, Ko B, Baumeister P, Swenson S, Costa F, Markland F, Stiles C, Patterson JB, Bates SE and Lee AS: Vascular targeting and antiangiogenesis agents induce drug resistance effector GRP78 within the tumor microenvironment. Cancer Res *65(13)*: 5785-5793, 2005.

27 Dimberg LY, Dimberg AI, Ivarsson K, Stromberg T, Osterborg A, Nilsson K, Oberg F and Wiklund HJ: Blood *106(4)*: 1346-1354, 2005.

28 Dunleavy K, Hakim F, Kim HK, Janik JE, Grant N, Nakayama T, White T, Wright G, Kwak L, Gress R, Tosato G and Wilson WH: B-cell recovery following rituximab-based therapy is associated with perturbations in stromal derived factor-1 and granulocyte homeostasis. Blood *106(3)*: 795-802, 2005.

29 Miyake K, Inokuchi K, Miyake N, Dan K and Shimada T: Antiangiogenic gene therapy of myeloproliferative disease developed in transgenic mice expressing p230 bcr/abl. Gene Ther *12*: 541-545, 2005.

30 Servida F, Soligo D, Delia D, Henderson C, Brancolini C, Lombardi L, Deliliers G and Lambertenghi T: Sensitivity of human multiple myelomas and myeloid leukemias to the proteasome inhibitor I. Leukemia *19*: 2324-2331, 2005.

31 Colla S, Morandi F, Lazzaretti M, Rizzato R, Lunghi P, Bonomini S, Mancini C, Pedrazzoni M, Crugnola M, Rizzoli V and Giuliani N: Human myeloma cells express the bone regulating gene Runx2/Cbfa1 and produce osteopontin that is involved in angiogenesis in multiple myeloma patients. Leukemia *19*: 2166-2176, 2005.

32 Van de Donk NWCJ, Lokhorst HM and Bloem AC: Growth factors and antiapoptotic signaling pathways in multiple myeloma. Leukemia *19*: 2177-2185, 2005.

33 Paris F, Sai-Maurel C, Faivre-Chauvet A, Thedrez P, Thomaret P, Barbet J, Chatal JF and Kraeber-Bodere F: Toxicity and efficacy of thalidomide associated with radioimmunotherapy in medullary thyroid cancer. Cancer Biother Radiopharm *19*: 516, 2004.

34 Schlenzka J, Moehler TM, Kipriyanov SM, Kornacker M, Benner A, Bahre A, Stassar MJJG, Schafer HJ, Little M, Goldschmidt H and Cochlovius B: Combined effect of recombinant CD19xCD16 diabody and thalidomide in a preclinical model of human B cell lymphoma. Anti-Cancer Drugs *15*: 915-919, 2004.