Eur J Cancer Clin Oncol, Vol. 19, No. 8, pp. 1127–1131, 1983.
Printed in Great Britain.

0277-5379/83$3.00+0.00

Tumour Control following Single-dose Irradiation of a Human Melanoma Xenograft*

EINAR K. ROFSTAD and TOR BRUSTAD

Norsk Hydro's Institute for Cancer Research and The Norwegian Cancer Society, The Norwegian Radium Hospital, Montebello, Oslo 3, Norway

Abstract—*A human malignant melanoma grown in the athymic nude mouse was exposed to single doses of X-rays and tumour control (TC) was studied. The* TCD_{50} *at* 70 days *post-irradiation was found to be* 27.4 ± 0.6 Gy. *This* TCD_{50} *is considerably lower than that predicted from the* in vitro *survival curve of cells from the melanoma irradiated* in vivo. *Studies also indicated that the tumour regrowth delay following large radiation doses possibly might be larger than indicated by the survival levels measured* in vitro. *Thus the radiation sensitivity of the melanoma measured* in vivo *appeared to be higher than that measured* in vitro. *This was probably not due to radiation damage to the vasculature only, as indicated by studies of the transplantability of irradiated tumours. An immune response by the nude mouse perhaps also contributed, as indicated by studies of the transplantability of the melanoma in whole-body-irradiated mice. If this was so, results from studies of the response to therapy of human tumours in the nude mouse, especially when tumour control is used as an endpoint, may not necessarily be representative for tumours in man.*

INTRODUCTION

SEVERAL methods for quantifying the radiation response of experimental rodent tumours have been developed [1]. The most commonly used endpoints are tumour regrowth delay, local tumour control and colony-forming ability *in vitro* following irradiation *in vivo*. By using different endpoints, it is possible to examine to what extent tumour regrowth delay or local tumour control is determined by inactivation of clonogenic cells, and to what extent other factors (e.g. radiation damage to the vasculature, immune response by the host) contribute. Studies of some rodent tumours have indicated a good correlation between these *in vivo* endpoints and colony-forming ability *in vitro* [2, 3], while studies of other tumours have indicated considerable discrepancies [4, 5].

The same endpoints are available for human tumour xenografts as for syngeneic rodent tumours. Few comparisons of results obtained with different endpoints have been reported [6, 7].

Accepted 8 March 1983.
*Financial support from The Norwegian Cancer Society, The Norwegian Research Council for Science and the Humanities and The Nansen Scientific Fund is gratefully acknowledged.

In previous work we have studied the radiation response of a human melanoma grown in the nude mouse using tumour regrowth delay and colony-forming ability *in vitro* as endpoints [7]. The purpose of the present work was to study the radiation response of the same xenograft using tumour control as the endpoint. The consistency between the data obtained with these three endpoints is discussed, and their potential to predict the radiation response of tumours in man is commented upon.

MATERIALS AND METHODS

Mice and tumour

Male NMRI/nu/nu/Han mice, kept under SPF (specific pathogen-free) conditions, were used in the present work.

The tumour (E.E. malignant melanoma), derived from a metastasis of a patient at The Norwegian Radium Hospital, was directly transplanted into nude mice. Histologically, the tumour tissue was composed of melanin-poor, atypical naevus cells growing in large spheres. Cells and nuclei varied greatly in size and shape. Numerous mitoses were seen. The tumour was grown serially in nude mice by implanting fragments, approximately 2 × 2 × 2 mm in size,

subcutaneously into recipient mice. Spontaneous regression of the tumour has never been observed.

Passages 40–46 of the tumour, implanted subcutaneously in the flank or in the leg of mice, were used in the present work. The tumour volume was about 200 mm^3 at the time of irradiation. Light microscopic examinations showed that the histological appearance of the serially transplanted xenograft was similar to that of the original tumour in the patient.

Irradiation procedure

Non-anaesthetized, normally breathing mice were irradiated locally at a dose rate of 5.1 Gy/min by using a 'Stabilipan' X-ray unit, operated at 220 kV and 20 mA, with 0.5 mm Cu filtration. A 15 × 15-mm hole through a 2-cm-thick lead block served as the beam-defining aperture. During exposure the mice were placed in specially made, thin-walled Perspex tubes with a hole in the cranial end through which they could breathe freely. A piston in the tail end positioned the mice firmly in the tubes. A hole was cut in each tube, either half-way up the tube or at the lower part, through which the flank tumours or the tumour-bearing legs protruded. To ensure uniform doses throughout the tumour volume, the mice were turned 180° halfway through each irradiation. The tumours were exposed to single doses in the range 17.5–35.0 Gy. Each group consisted of 20–22 mice. The irradiation was performed behind the SPF barrier.

Evaluation of tumour control

Denekamp [1] has indicated that tumours should usually be followed for at least 90 days before evaluation of tumour control. The observation period of the present melanoma was limited by the time an adequate number of mice appeared healthy. Thus the tumours were examined twice weekly and scored as being controlled if regrowth was not observed within 70 days post-irradiation. Only mice which appeared healthy until day 70, or had died before this time due to a large recurrent tumour, were included in the analysis. The TCD_{50}, i.e. the radiation dose required to control 50% of the tumours, was computed by logit analysis [8].

RESULTS

The TCD_{50} ± standard error of the mean for tumours growing in the leg was found to be 27.4 ± 0.6 Gy (Fig. 1). The regrowth of the tumours which were not controlled after exposure to 25.0–30.0 Gy was initiated between 2 and 8 weeks after irradiation (Fig. 2). Morphologically intact tumour cells were not observed when the tumour-bearing legs of mice scored as cured were subjected to histological examinations. In spite of the limited life span of the mice, 3 mice exposed to 25.0 Gy and 5 mice exposed to 27.5 Gy remained healthy for 6 months after irradiation, and tumour regrowth was never observed beyond day 70 in mice scored as cured. This indicates that a period of 70 days post-irradiation was sufficient for an accurate determination of the TCD_{50} of the present melanoma. The TCD_{50} of tumours growing in the flank could not be determined, due to severe radiation damage to the intestine at doses above 27.5 Gy. However, tumour control data for lower doses suggest that tumours growing in the flank were slightly more radioresistant than those growing in the leg (Fig. 1). The higher resistance of the flank tumours was not due to differences in tumour size.

The observed TCD_{50} was surprisingly low (cf. Discussion section), and in order to shed light on the underlying mechanisms, two types of experiments were carried out.

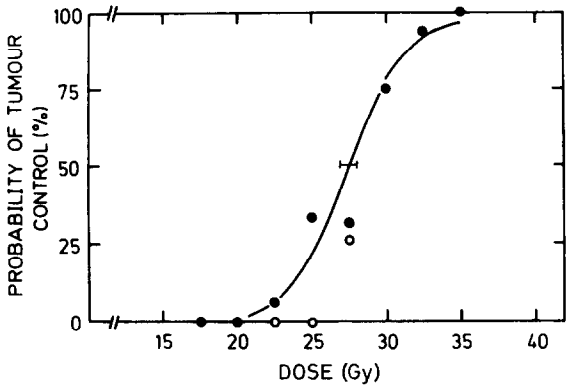

Fig. 1. Probability of tumour control plotted against radiation dose for a human melanoma xenograft growing in the leg (closed circles) or in the flank (open circles) of nude mice. Each point is based on 15–20 tumours. The horizontal bar represents the standard error of the mean at the TCD_{50} level.

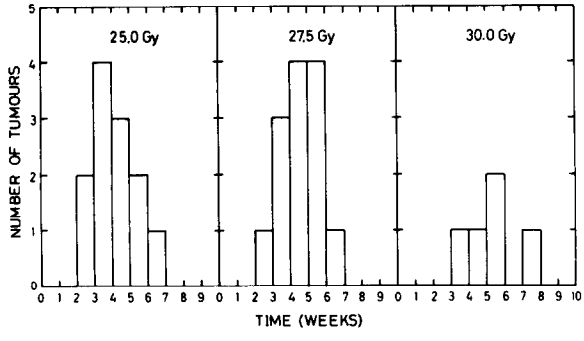

Fig. 2. Time distributions of observed recurrences following exposure to 25.0, 27.5 and 30.0 Gy for a human melanoma xenograft growing in the leg of nude mice. The columns of the histograms represent the number of tumours which recurred each week after irradiation. After 25.0 Gy 12 of 18 tumours recurred, after 27.5 Gy 13 of 19 tumours recurred and after 30.0 Gy 5 of 20 tumours recurred.

Firstly, the transplantability of irradiated tumours was studied. Tumours growing in the flank were irradiated, left *in situ* for 24 hr to allow for repair of potentially lethal damage (PLD), and then excised and cut into fragments, approximately 2 × 2 × 2 mm in size. The fragments were implanted subcutaneously into the flanks of recipient mice and examined with respect to growth. Growth was observed in nearly 100% of the implants from tumours exposed to 5.0 and 10.0 Gy and only rarely in those from tumours exposed to 20.0, 25.0 and 30.0 Gy (Table 1).

Table 1. Fraction of tumours developed from implants from individual, irradiated tumours

Dose (Gy)	Tumour No. I	II	III
0	9/9	12/12	9/10
5	12/12	10/10	10/10
10	7/8	11/11	10/10
15	5/10	2/9	6/9
20	3/9	0/11	1/10
25	0/8	0/7	0/11
30	0/8	0/9	0/10

The mice were examined up to 100 days post-implantation.

Secondly, the transplantability of the melanoma in whole-body-irradiated mice was studied. Tumour fragments, approximately 1 × 1 × 1 mm in size, were implanted subcutaneously into mice which had received 3.5 Gy whole-body irradiation 24 hr before implantation [9] and into unirradiated mice. In irradiated mice 100% (9/9) of the implants developed tumours, while in unirradiated mice only 75% (8/12) developed tumours. The growth rate of the tumours in unirradiated and in whole-body-irradiated mice was not significantly different (Fig. 3). However, the data in Fig. 3 suggest that the latency period was a few days longer in unirradiated than in whole-body-irradiated mice.

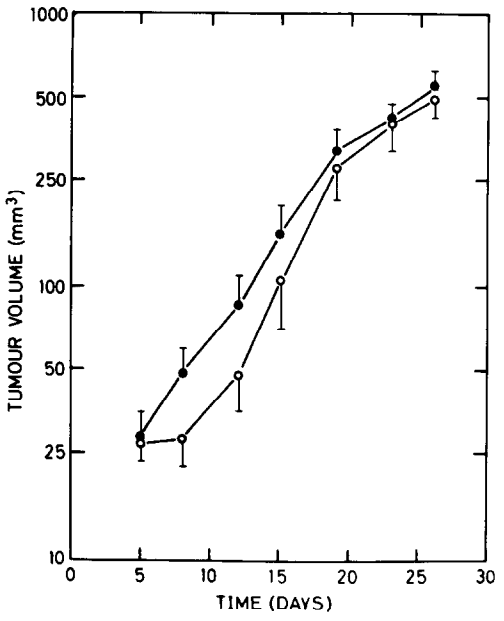

Fig. 3. Tumour volume plotted against time after implantation for a human melanoma xenograft growing in the flank of nude mice. Tumour fragments were implanted in unirradiated mice (open circles) and in mice exposed to 3.5 Gy whole-body irradiation 24 hr before implantation (closed circles). Two perpendicular tumour diameters were measured with callipers and the tumour volumes were calculated as $V = \frac{1}{2} \cdot ab^2$, *where a and b are the longest and the shortest diameter respectively. The curves are based on 8 tumours in unirradiated mice and 9 tumours in whole-body-irradiated mice. Standard errors of the mean are indicated by vertical bars.*

DISCUSSION

In a previous study with the same melanoma [7], tumours growing in the flank were irradiated *in vivo* with ^{60}Co γ-rays before clonogenic cell inactivation was assayed *in vitro* in soft agar. When tumours irradiated in normally breathing mice were left *in situ* for 14 hr after irradiation to allow for PLD-repair, the exponential portion of the survival curve was characterized by $D_0 = 2.90 \pm 0.25$ Gy and $n = 4 \pm 2$. Assuming that (a) the survival curve at high doses has the form $S = n \exp(-D_0/D)$, (b) the tumours can recur from one cell and (c) N is the number of clonogenic cells in the tumours, the TCD_{50} is, according to McNally and Sheldon [5], given by:

$$TCD_{50} = D_0 \cdot \left[\ln n - \ln \frac{\ln 2}{N}\right].$$

Theoretical TCD_{50} values for the melanoma, based on the survival curve parameters mentioned above, were calculated as a function of N using this equation (Fig. 4). As shown in the figure, the TCD_{50} measured in the present work suggests that the number of clonogenic cells in the tumours is in the range 10^3–10^4. Since the *in vitro* survival curve was established from tumours in the flank irradiated with ^{60}Co γ-rays and the TCD_{50} from tumours in the leg irradiated with X-rays, the results cannot be compared directly. However, even though the measured TCD_{50} is corrected for the higher resistance of tumours in the flank suggested by Fig. 1 (TCD_{50} was assumed not to be higher than 32 Gy) and for the difference in RBE between ^{60}Co γ-rays (~0.86) [10] and X-rays (close to 1), the number of clonogenic cells in the tumours can probably not be estimated to be higher than 10^5 (Fig. 4). Since the tumours consist of 10^8–10^9 cells/g tumour weight, the real number of clonogenic cells per tumour (V ~200 mm³) is probably higher than 10^7, corresponding to a theoretical TCD_{50} higher than 50 Gy (Fig. 4).

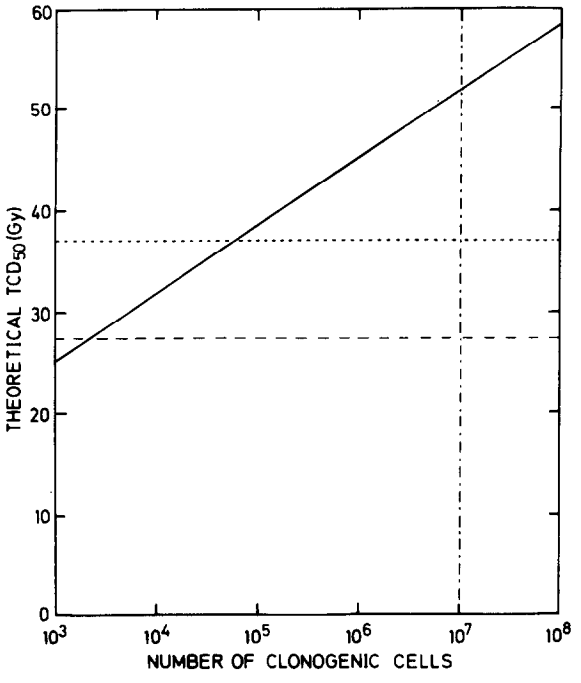

Fig. 4. Theoretical TCD_{50} for a human melanoma xenograft, based on in vitro *measurements of clonogenic cell inactivation following irradiation* in vivo, *plotted against number of clonogenic cells per tumour (———). TCD_{50} measured for tumours growing in the leg (— — —). This TCD_{50} corrected for the higher resistance of tumours growing in the flank and for the difference in RBE between ^{60}Co γ-rays and X-rays (see text) (· · · · ·). Estimate of the real number of clonogenic cells per tumour (— · —).*

Consequently, the measured TCD_{50} for the melanoma is considerably lower than that predicted from the *in vitro* survival curve, suggesting that tumour control can be obtained without all cells being inactivated by the radiation.

Results from previous studies [7, 11] also suggest that the tumour regrowth delay of the melanoma does not appear to be determined directly by the fraction of surviving cells as measured *in vitro*. Figure 5 shows the tumour regrowth delay as a function of the fraction of surviving cells when allowing for PLD-repair. The data are based on tumours in the flank of mice irradiated with ^{60}Co γ-rays [7]. If the regrowth delay depended on the fraction of surviving cells only, it would be expected that the extrapolation of the curve would intersect the abscissa close to the origin, since $n = 4 \pm 2$ for the *in vitro* survival curve. The extrapolation of the curve in Fig. 5 intersects the abscissa relatively far from the origin, probably mainly because the rate of regrowth of the melanoma decreases with increasing radiation dose [7, 11]. Consequently, studies using tumour regrowth delay as the endpoint also suggest that the radiation response of the melanoma *in vivo* is probably higher for

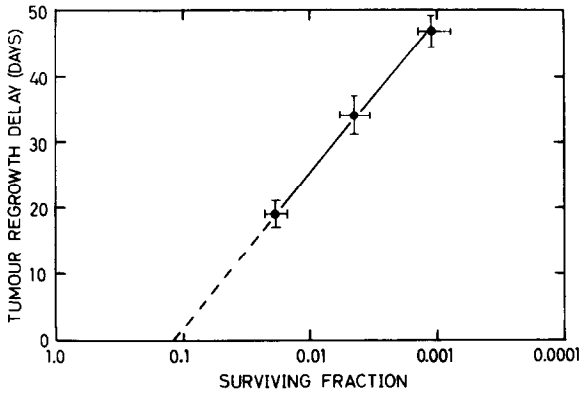

Fig. 5. Tumour regrowth delay plotted against fraction of surviving cells measured in vitro *following irradiation* in vivo *for a human melanoma xenograft. The bars represent standard errors of the mean. Tumour regrowth delay was defined as the time from the day the tumours were irradiated to the day they had regrown to their original size. Details in the experimental procedure have been reported previously* [7, 11].

large radiation doses than that indicated by the fraction of surviving cells as measured *in vitro*.

Although the vasculature of the melanoma is considerably damaged by the present radiation doses (manuscript in preparation), the results presented in Table 1 suggest that the relatively high radiation sensitivity *in vivo* is not due to radiation damage to the vasculature only. Tumour fragments approximately $2 \times 2 \times 2$ mm in size (*ca.* 10^6–10^7 cells) from tumours exposed to 25.0 Gy were not able to form tumours when implanted into the flanks of mice with intact vasculature. Since the fraction of surviving cells measured *in vitro* following exposure to 25.0 Gy is about 10^{-3} [7], other factors than clonogenic cell inactivation and radiation damage to the vasculature probably also contribute to the radiation response of the melanoma *in vivo*.

The nude mouse has been shown to exhibit an unusually high level of natural killer cells (NK cells) compared with conventional mice [12]. It has also been shown that certain lymphoid cells in the nude mouse express surface antigens characteristic of mature T lymphocytes [13]. The NK cells and the 'T-like' cells may interact with cells of human tumours and hence enhance the response to therapy of xenografts. Thus some xenografts have been shown to be locally controlled when treated with relatively low doses of chemotherapeutic agents, and Steel and Peckham [14] have suggested that this may be due to participation of host defence mechanisms. The present study indicated that the transplantability of the melanoma was lower and the latency period slightly longer in unirradiated than in whole-body-irradiated mice (Fig. 3). Thus an immune response by the host, in addition to radiation damage to the vasculature, may perhaps explain

the relatively large radiation response *in vivo* observed for the melanoma. If this is so, results from studies of the therapeutic response of human tumours in the nude mouse, especially when tumour control is used as the endpoint, may not necessarily be representative for tumours in man.

REFERENCES

1. DENEKAMP J. Experimental tumor systems: standardization of endpoints. *Int J Radiat Oncol Biol Phys* 1979, **5**, 1175–1184.
2. REINHOLD HS, DE BREE C. Tumour cure rate and cell survival of a transplantable rat rhabdomyosarcoma following X-irradiation. *Eur J Cancer* 1968, **4**, 367–374.
3. BARENDSEN GW, BROERSE JJ. Experimental radiotherapy of a rat rhabdomyosarcoma with 15 MeV neutrons and 300 kV X-rays—I. Effects of single exposures. *Eur J Cancer* 1969, **5**, 373–391.
4. ROCKWELL S, KALLMAN RF. Cellular radiosensitivity and tumor radiation response in the EMT-6 tumor cell system. *Radiat Res* 1973, **53**, 281–294.
5. MCNALLY NJ, SHELDON PW. The effect of radiation on tumour growth delay, cell survival and cure of the animal using a single tumour system. *Br J Radiol* 1977, **50**, 321–328.
6. GUICHARD M, CHAVAUDRA N, MALAISE EP. Tumour growth delay and cell survival as endpoints for studying the fraction of hypoxic cells in a human melanoma transplanted into nude mice. *Int J Radiat Biol* 1980, **38**, 349–353.
7. ROFSTAD EK. Radiation response of the cells of a human malignant melanoma xenograft. Effect of hypoxic cell radiosensitizers. *Radiat Res* 1981, **87**, 670–683.
8. SUIT HD, SHALEK RJ, WETTE R. Radiation response of C3H mouse mammary carcinoma evaluated in terms of cellular radiation sensitivity. In: *Cellular Radiation Biology*. Published for The University of Texas M.D. Anderson Hospital and Tumor Institute. Baltimore, MD, Williams and Wilkins, 1965, 514–530.
9. PREHN LM, OUTZEN HC. Primary tumor immunity in nude mice. *Int J Cancer* 1977, **19**, 688–691.
10. HALL EJ. The relative biological efficiency of X rays generated at 220 kVp and gamma radiation from a cobalt 60 therapy unit. *Br J Radiol* 1961, **34**, 134.
11. ROFSTAD EK, BRUSTAD T. The radiosensitizing effect of metronidazole and misonidazole (Ro-07-0582) on a human malignant melanoma grown in the athymic mutant nude mouse. *Br J Radiol* 1978, **51**, 381–386.
12. HERBERMAN RB. Natural cell-mediated cytotoxicity in nude mice. In: FOGH J, GIOVANELLA BC, eds. *The Nude Mouse in Experimental and Clinical Research*. New York, Academic Press, 1978, Vol. I, 135–166.
13. MACDONALD HR. Phenotypic and functional characteristics of 'T-like' cells in nude mice. *Exp Cell Biol* 1982, **50**, 305.
14. STEEL GG, PECKHAM MJ. Human tumour xenografts: a critical appraisal. *Br J Cancer* 1980, **41** (Suppl. IV), 133–141.