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Human tumor models in the severe combined immune deficient (*scid*) mouse

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Abstract *Purpose*: To test a number of established human tumor cell lines and early passage breast cancer (UACC2150) and melanoma cells (UACC1273) for growth in the *scid* mouse and the tumors' response to conventional chemotherapeutic drugs. *Methods*: Established melanoma (A375, C81-61), colon (SW480), lung (A549), lymphomoblastoid leukemia (LCL-B), promyelocytic leukemia (HL60), prostate (PC-3, DU145), and breast (MCF7) cell lines were injected at subcutaneous (s.c.), intraperitoneal (i.p.), or mammary fat pad (MFP) sites. Tumor volume growth curves and survival curves were established for the various tumor cell lines. Carmustine (BCNU), cisplatin (CDDP), cyclophosphamide (CPA), doxorubicin, dacarbazine (DTIC), tamoxifen and vincristine were injected s.c. or i.p.. The chemotherapeutic drug effects on tumor volumes and survival were determined. *Results*: Tumor growth occurred with each cell type. After i.p. injection, 90% mortality occurred within 26 to 60 days except for the early passage melanoma cell line UACC1273 with which mortality occurred within approximately 90 days. In the MCF7 breast model, treatment with tamoxifen ($P < 0.001$) and CPA ($P < 0.0001$) resulted in significant tumor growth delay compared with control groups. BCNU and CDDP resulted in significant tumor growth delays relative to control in SW480 colon cancer ($P < 0.0014$) and A375 melanoma ($P < 0.0001$) models, respectively. CPA and doxorubicin improved survival in the HL60 leukemia model ($P = 0.0018$). *Conclusions*: These *scid* mouse human tumor models appear to reflect the clinical situation in that clinically active chemotherapeutic drugs are similarly active in the *scid* mouse models. Therefore, the *scid* mouse models may be useful for testing new chemotherapeutic agents against various human cancer types.

Key words *Scid* mouse · Cancer model · Chemotherapy · Human tumors

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Introduction

In 1983 Bosma et al. reported a severe combined immune deficient (*scid*) syndrome in balb/c mice [1]. A colony of mice deficient in mature B and T lymphocytes was established by selective breeding. Germ line DNA segments encoding immunoglobulin (Ig) and T lymphocyte antigen receptor molecules fail to undergo rearrangement in the *scid* mouse, which thus lacks functional antigen receptors [11]. A homozygous mutation in the *scid* mouse has been mapped to chromosome 16 and results in a defect in the rearrangement of genes that code for antigen-specific receptors on B cells and T cells [13]. The *scid* mouse may occasionally express mature B and T lymphocytes indicating "leakiness" for the *scid* defect [2, 3]. The defect does not affect natural killer, myeloid, and antigen-presenting cells [13]. Harrington et al. have demonstrated that DNA end joining (integration of linear DNA) is also decreased in *scid* mouse fibroblasts [6]. The defect in V(D)J gene segment recombination in the *scid* mouse resembles the recently described recombination defect in B lymphocyte-negative *scid* patients [12].

The *scid* mouse will support local and metastatic growth of various human tumor cells. Human solid tumors shown to grow in the *scid* mouse include breast cancer [18], lung cancer [10], and malignant melanoma [7, 9]. The objectives of our studies were to develop *scid*

mouse models that would accept human tumor xenografts and reflect clinical results with regard to response to chemotherapy. These human tumor models could then be used for in vivo testing of new chemotherapeutic drugs.

Materials and methods

Scid mouse colony and procedures

A *scid* mouse colony was developed at the University of Arizona using original *scid* (C.B-17 *scid*/*scid*) and (Balb/cByJSmn-*scid*/J) obtained from Jackson Laboratory (Bar Harbor, Me.). The mice were housed in microisolator cages (Allentown Caging Equipment Company, Allentown, N.J.) and maintained under specific pathogen-free conditions. The mice ate LM45 5% fat, autoclavable pellets (Tekland Premier, Madison, Wis.) and drank ultraviolet-irradiated water. Every month mice were screened by ELISA serology for mycoplasma, mouse hepatitis virus, pinworms, and Sendai virus and tested negative.

Female and male mice 6–8 weeks of age were bled (200 µl) by retroorbital puncture in order to screen for the presence of mouse Ig using an ELISA [5]. Only mice with Ig levels ≤ 5 µg/ml were used for the experiments. Mice were weighed once weekly. *Scid* mice injected with hormone-dependent breast cancer cell lines were implanted subcutaneously (s.c.) with 17β-estradiol (Innovative Research of America, Toledo, Ohio) 1 day prior to tumor cell injection and every 21 days thereafter. Subcutaneous tumor cell injections were given on the mouse's lower right flank in a total volume of 200 µl intraperitoneal (i.p.) tumor cell injections (200 µl) were given in the right side of the abdomen and i.p. drug injections (200 µl) were placed on the left side of the abdomen. Early passage breast cancer tumor cells (200 µl) for mammary fat pad (MFP) studies were injected s.c. into the lower left MFP. Following development, the volume (cm^3) of s.c. and MFP tumors were estimated in accordance with the formula (tumor $width^2$ × tumor length/2) [15] while mice with i.p. tumors were followed for survival. The mice were sacrificed by cervical dislocation after anesthesia with ketamine HCl 60–70 mg/kg (Aveco Co., Fort Dodge, Iowa) and xylazine 5–7.5 mg/kg (Lloyd Laboratories, Shenandoah, Iowa).

Tumor cells

Early passage UACC1273 melanoma (derived from an untreated left axillary lymph node in a 54-year old man), and established lung (A549), colon (SW480), lymphomoblastoid (LCL-B), leukemia (HL60), prostate (PC-3, DU145), melanoma (C81-61, A375) and breast (MCF7) cell lines were grown in RPMI-1640 medium (Sigma, St. Louis, Mo.) supplemented with 10% fetal bovine serum (Gibco, Grand Island, N.Y.), 1% L-glutamine (Fisher, Phoenix, Ariz.), and 1% penicillin streptomycin (Sigma) and maintained in a humidified atmosphere of air containing 5% CO_2 at 37 °C. Cell lines were grown to 95–100% confluence. Trypsin (1 ml trypsin solution/5 ml of Hanks balanced salt solution, HBSS) was added to detach adherent cells. Cells (90–100% viability) were counted and resuspended at a concentration of 10×10^6 cells/200 µl sterile saline.

Early passage (less than ten in vitro subcultures from biopsy sample) breast cells (UACC2150) were derived from a malignant pleural effusion in a 46-year-old woman with breast cancer. The karyotype obtained from passage 1 showed a range of chromosomes counts from 41 to 83 and bimodal distribution at 49–52 and 64–68. The stemline karyotype was 49–52, X, −X, inv(1)(p34p22), +der(2)t(1;2)(q10;q13), add(3)(p11), +6, +del(6)(q14q16), +7, i(8)(q10), −9, i(14)(q10), −16, add(17)(p11), −18, +20, +20, −21, −21, +5mar [cp]. The patient's primary tumor was estrogen receptor (ER) and progesterone (PR) positive. UACC2150 cells were her-2-neu positive and epidermal growth factor receptor (EGFr) negative as measured using a human *neu* quantitative and human epidermal growth factor receptor quantitative ELISA, respectively (Oncogene Science, Uniondale, N.Y.). UACC2150 early passage cells were grown in M41 medium (L15 medium, American Biorganics, Niagara Falls, N.Y.), containing EGF (Harlan Bioproducts, Indianapolis, Ind.), and collagen-coated matrix (ICN, Costa Mesa, Calif.) and maintained in capped flasks at 37 °C. Cells were counted and resuspended at concentrations of 5, 10 or 20×10^6 cells/100 µl matrigel (Becton Dickinson, Bedford, Md.) and 100 µl sterile saline and used for experiments.

Chemotherapeutic agents

The chemotherapeutic drugs were prepared and injected in approximately 1 h. Table 1 shows the dose, route of injection, schedule, diluent and source for each drug [4]. The drug doses were considered to be the maximally tolerated doses (MTDs) in the scid mouse because they do not invoke a weight loss of more than 20% of total weight.

Statistical analysis

The natural log of the relative tumor volumes was used for statistical analysis, because it resulted in values that were more normally distributed. Comparisons between treated and control mice across time were performed using repeated measures analysis of variance. Two aspects of the growth curves were compared. The first was whether or not the curves had the same profile (shape) across time while the second assessed the magnitude of the difference between groups for those with similar profiles. To determine whether the

Table 1 Optimal drug doses and schedules

Drug	Dose	Route of injection	Schedule[a]	Diluent	Source	Tumor models
BCNU	20 mg/kg	i.p.	Day 1	Saline	Bristol-Meyers Princeton, N.J.	Colon
CDDP	3.5 mg/kg	i.p.	Days 1, 5, 9	Water	Bristol-Meyers, Princeton, N.J.	Melanoma
CPA	265 mg/kg	i.p.	Day 1	Water	Sigma, St. Louis, Mo.	Breast, Leukemia
Doxorubicin	2–6.7 mg/kg	i.p.	Days 1, 5, 9	Water	Sigma, St. Louis, Mo.	Leukemia
DTIC	80 mg/kg	i.p.	Days 1–9	0.05 *N* HCl	Sigma, St. Louis, Mo.	Melanoma
17 β-estradiol	0.25 mg pellet	s.c.	Every 3 weeks	None	Innovative Research of America, Toledo, Ohio	Breast
Tamoxifen	50 mg/kg	s.c.	Days 1–19	Peanut oil	Sigma, St. Louis, Mo.	Breast
Vincristine	0.5 mg/kg	i.v.	Days 1, 5	Saline	Sigma, St. Louis, Mo.	Leukemia

[a] Various tumor cells were injected on day 0

profiles were the same, the statistical significance of the groups-by-day interaction term was assessed. When this term was significant, it suggested that the growth rate profile (shape) differed between the two groups. In these cases the overall difference between the treated and control groups was then assessed. In the survival curve analysis significant differences in survival were evaluated using a log-rank test with a Bonferroni adjustment for multiple comparisons.

Results

Each of the ten different established cell lines and the early passage breast and melanoma cells engrafted in the *scid* mouse. Colon, lung, lymphomoblastoid, leukemia, prostate and melanoma cell lines engrafted at the s.c. location and showed progressive increases in s.c. tumor volume over time with notable growth after day 10 (Fig. 1A,B). To correct for minor variations in lag time, data were normalized by dividing each individual mouse's tumor volumes, over time by the mouse's first measurable day's tumor volume which was not always the same day as other mice, so that all tumors started with the same relative volume. The normalized mean relative tumor volume curve was then determined, allowing statistical analysis of the entire growth curve for comparison of treatment versus control groups. After i.p. tumor cell injection in most tumor types, 90% mortality occurred within 26 to 60 days (Fig. 2A,B). However, the early passage melanoma cells (UACC1273) grew more slowly, and 90% lethality occurred within approximately 90 days.

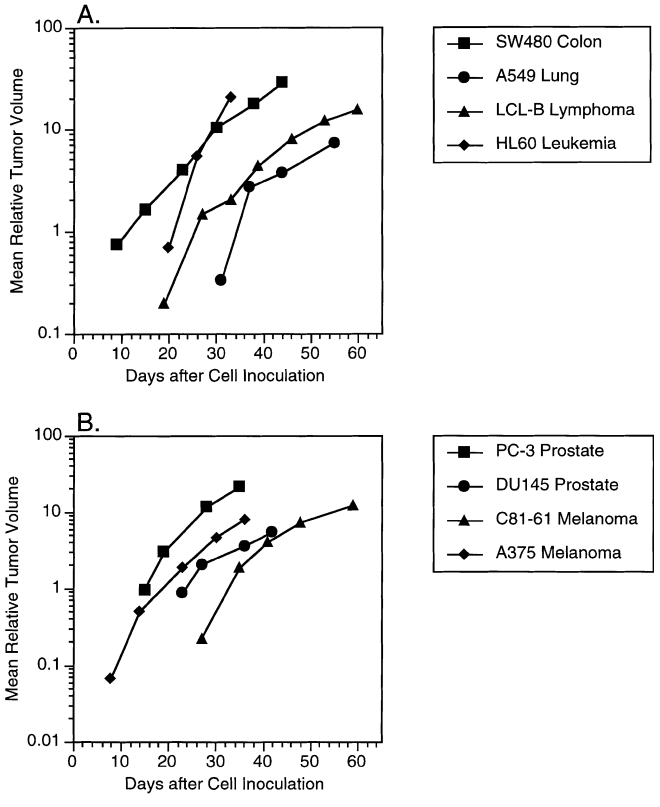

Fig. 1A,B Mean relative tumor volume curves for groups of *scid* mice injected with various human tumors ($n = 15$ per tumor type and repeated two or more times depending on tumor type). The mean relative tumor volume is derived by calculating the mean of the tumor volumes expressed relative to the first day of tumor measurement for each individual *scid* mouse (i.e. for each individual *scid* mouse all subsequent tumor volumes are divided by the value of the first tumor volume). The first tumor volume measurement for each individual mouse ranged from 0.032 to 0.405 cm^3

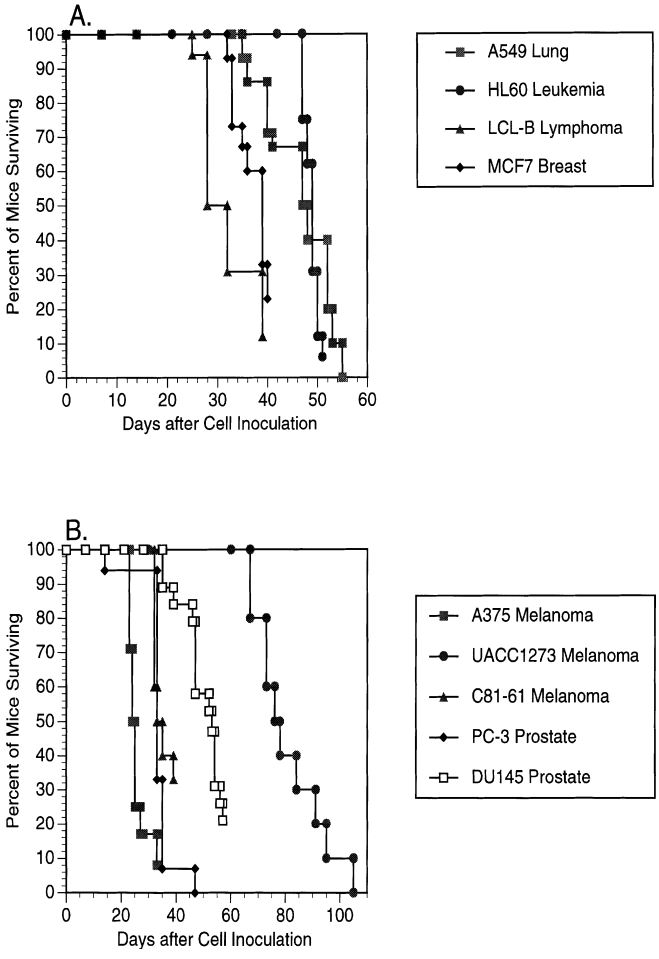

Fig. 2A,B Survival curves for untreated *scid* mice bearing various human tumors i.p. ($n = 6$ to 19 per tumor type and repeated two or more times depending on tumor type)

Figure 3 shows the results for UACC2150, an early passage breast cancer cell line, growing orthotopically in MFP. UACC2150 cells have a doubling time of approximately 7 days in vitro. Progressive growth occurred with each cell inoculum from 5 to 20 $\times$ 10^6 cells with no statistical difference between the curves. The levels of her-2-neu and EGFr in these cells were 9.6 and 0.04 fmol/μg, respectively.

Figure 4A,B shows MCF7 breast cancer models treated with tamoxifen and cyclophosphamide (CPA). There was a significant group-by-day interaction for tumor volume using both tamoxifen and CPA ($P < 0.001$ and $P < 0.0001$, respectively). The tamoxifen group's tumor volume curve increased at a slower rate after day 15. The tumor growth curves for SW480 colon cancer control and carmustine (BCNU)-treated groups (Fig. 4C) had the same shape across time. The A375

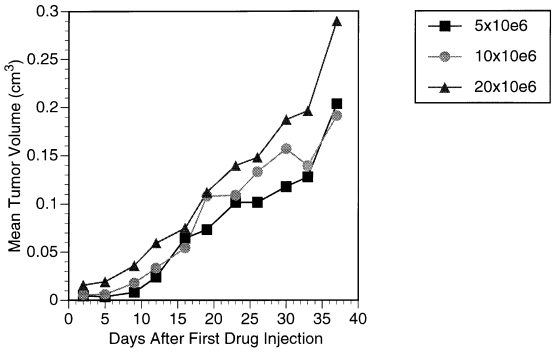

Fig. 3 Tumor growth curve for UACC2150 early passage breast cancer cells injected in the MFP ($n = 8$ per cell number). First tumor volume measurements ranged from 0.004 to 0.018 cm^3

melanoma tumor growth curves of the control versus cisplatin (CDDP)-treated groups had the same general shape over time, but the CDDP curve was significantly different from the control ($P < 0.0001$, Fig. 4D).

In the HL60 leukemia model, survival was enhanced relative to control after treatment with CPA ($P = 0.0018$) and doxorubicin ($P = 0.0018$, Fig. 5A). In the A375 melanoma model (Fig. 5B) there was a significant difference in survival for the CDDP-treated group compared with both the control and dacarbazine (DTIC)-treated groups ($P = 0.0003$).

Discussion

The objective of these experiments was to establish *scid* mouse human tumor models and evaluate correlation with clinical chemotherapy responsiveness. Therefore, we first attempted to obtain engraftment of various tumor types in the *scid* mouse including established cell lines and early passage cells. The early passage breast (UACC2150) and melanoma cells (UACC1273), which grew progressively in the *scid* mouse, should have greater clinical relevance because they have less than ten in vitro subcultures since being obtained from their patients of origin. Established cell lines with innumerable subcultures have undergone extensive selection for in vitro growth and have undoubtedly lost many of the characteristics present in the original patient biopsy.

We tested whether chemotherapeutic agents active clinically for specific tumor types were active in the *scid* mouse human tumor models. The evaluation of new chemotherapeutic agents has been stalled by the lack of reproducible animal models which have relevance to the clinical situation and give results in a relatively short time. Previous attempts to establish human xenografts in the nude mouse have shown the difficulty of obtaining stable xenografts and have raised questions about their clinical relevance. Xie et al. have shown greater metastatic capacity for human bladder cancer, colon cancer and malignant melanoma cells in *scid* (96% lung metastasis incidence) versus nude (27%) mice [17]. In addition, Taylor-Papadimitriou et al. have found that cell lines (e.g. MCF7) do not grow quickly in the nude mouse and that established in vivo tumors are difficult to obtain even when supported with hormone implants [16]. Kawata et al. have found that *scid* mouse models have distinct advantages over nude mouse models for growing human B leukemia/lymphoma cells including 100% transplantation of tumor with lower cell numbers

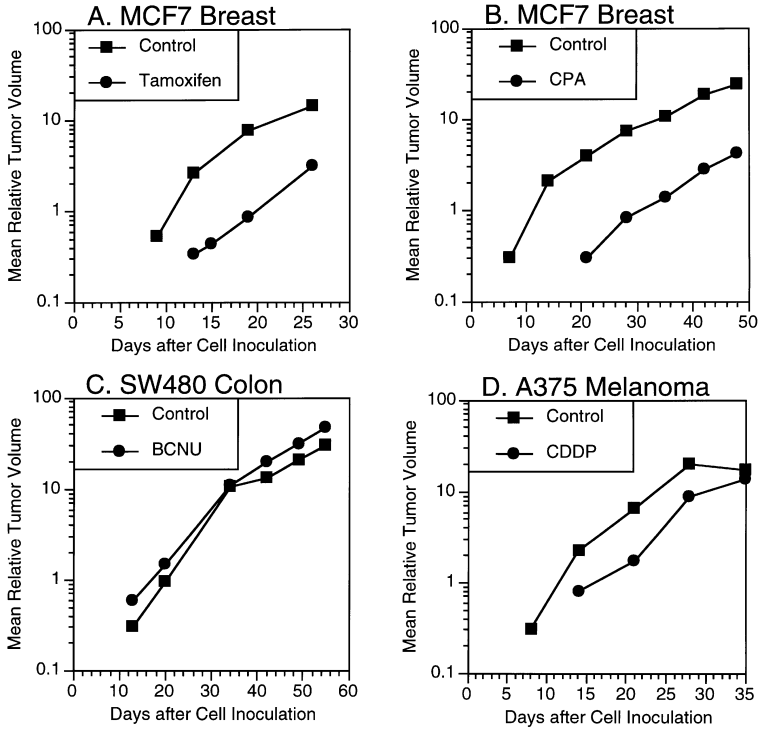

Fig. 4A–D Treatment results (measured as tumor growth delay) for *scid* mice bearing a clinically chemotherapy-responsive tumor (breast) versus a clinically less-responsive tumor (colon or melanoma). Tumor cells were injected s.c. while drugs were administered at a different s.c. site (tamoxifen) or i.p. (CPA, BCNU, CDDP). There were nine or ten mice per experimental group. The tumor volume in control mice ranged from 0 to 0.171 cm^3 and in drug-treated mice from 0 to 0.032 cm^3 on the first day of measurement

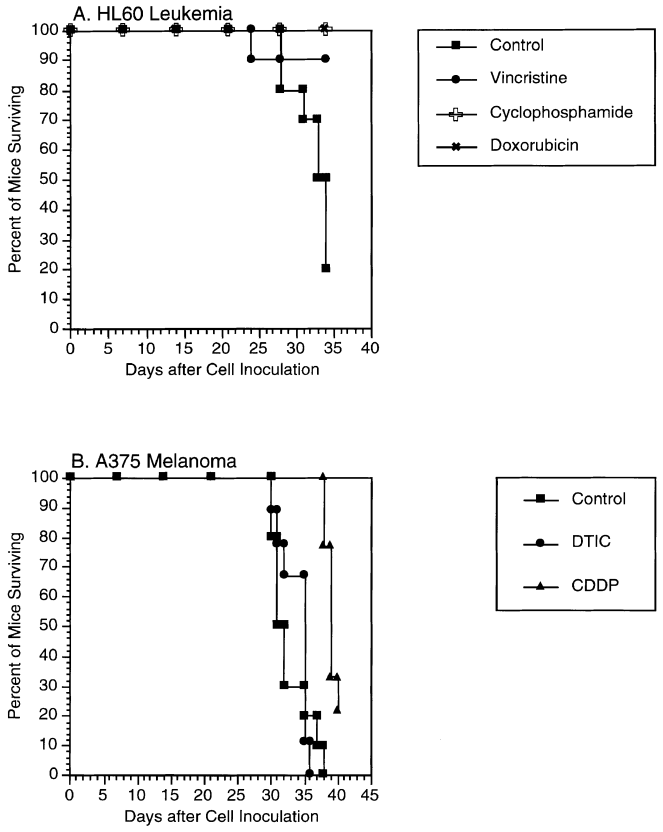

Fig. 5A,B Survival curves for *scid* mice receiving i.p. injections of HL60 leukemia (**A**) or A375 melanoma (**B**) and treated with i.v. vincristine or i.p. CPA, doxorubicin, DTIC or CDDP. There were nine or ten mice per experimental group

and greater dissemination [8]. Because the *scid* mouse has a more profound immune deficiency than the nude mouse, it allows more consistent and extensive growth of human tumor cells. Current evidence indicates that the scid mouse may be more permissive of human tumor growth and may have greater clinical relevance than the nude mouse even though several of these cell lines can establish tumors in the nude mouse.

Previous mouse animal models used to screen new therapeutic modalities are poorly predictive for antitumor activity in human clinical trials of patients with solid tumors. Taetle et al. have performed extensive studies of human melanoma xenografts in the nude mouse. They conclude that the most important biological variable for predicting in vivo drug responsiveness is in vitro tumor growth rate [14, 15]. In the current studies, the *scid* mouse supported growth of various established and early passage human tumor types after s.c., i.p., and MFP injection. Of note, our UACC2150 early passage breast cancer cells grew less vigorously in vitro than established breast cancer cell lines such as MCF7, yet in the *scid* mouse UACC2150 had substantial growth within 30 days at an orthotopic MFP site.

Drugs that are known to be clinically effective were efficacious in our *scid* mouse human tumor models. Specifically, breast cancer, which is responsive to tamoxifen and CPA in patients, similarly responded to these agents in the *scid* mouse. The *scid* mouse leukemia model also reflected clinical results by showing significantly reduced mortality in doxorubicin-treated leukemic mice. In contrast, colon cancer and melanoma are resistant to chemotherapy in the clinic, and this was again reflected in our *scid* mouse human tumor models. Our results suggest that the *scid* mouse models may be useful for in vivo testing of new chemotherapeutic agents against various human tumor types. Clinically relevant in vivo models for testing new anticancer drugs should yield significant benefits by saving patients from toxic drugs unlikely to be active clinically and by yielding a greater chance of discovering drugs which will be active in vivo. This would also result in economic benefit by limiting expensive clinical trials to drugs with a greater chance of being tolerable and active.

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