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Human Tumor Cloning: Feasibility and Clinical Correlations

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Summary. *The human tumor cloning system is a soft agar technique which allows the growth of human tumors in vitro. We report here our experience with culturing 2,365 patients' tumors in the system. Overall 1,844 (78%) have formed colonies in vitro. However, only 51% have formed ≥ 30 colonies per 500,000 cells plated. Despite the limitations of inadequate growth for some tumors there are a number of clinical applications for the system, which are reported here. These include: (1) Use of the system to predict for sensitivity of an individual patient's tumor to a particular chemotherapeutic agent; (2) screening new anticancer agents to predict for in vivo activity; 3) monitoring patients' bone marrows for tumor involvement; and (4) use of the number of colonies which form in the assay as a prognostic factor for survival. All of these clinical applications are in their infancy of development and will require carefully designed prospective trials to determine the final place of the human tumor cloning system in the practice of clinical oncology.*

Introduction

The development of a two-layer soft agar system by Hamburger and Salmon [3, 4] for growing a variety of human malignancies has stimulated a great deal of work with the system. One major area of effort has involved applications of the assay system to a variety of clinical situations.

In this paper the results of a large number of in vitro-in vito correlative studies are presented. This information should provide a basis for future clinical trial designs based on human tumor cloning data.

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Patients and Methods

After giving informed consent, patients undergoing diagnostic or therapeutic procedures for presumed or proven malignancies had part of their specimen sent for culturing in soft agar. A total of 14 hospitals in the San Antonio and surrounding areas (70-mile radius) have participated in the program.

Collection of Cells. Solid tumor or lymph nodes obtained immediately after surgery and after pathologic assessment were placed in transport medium containing McCoy's 5A + 10% heat-inactivated fetal calf serum + 1% penicillin/streptomycin solution (all from Grand Island Biological Company, Grand Island, NY). Upon receipt of these specimens in the laboratory (usually within 4–6 h after surgery) the tumors were mechanically dissociated under aseptic conditions. These solid tumors were minced with a scalpel, teased apart with needles, passed through 20-, 22-, and 25-gauge needles, and then washed by centrifugation as previously described [5]. Ascitic, pleural, and pericardial fluids obtained by standard clinical techniques were placed in sterile containers containing 100 units of preservative-free heparin per milliliter of malignant fluid. After centrifugation at 150 *g* for 10 min, the cells were harvested and washed twice in Hank's balanced salt solution with 10% heat-inactivated fetal calf serum (both obtained from Grand Island Biological Company, Grand Island, NY). Effusions contaminated with red blood cells were treated with a NH_4Cl-lysing buffer and then washed with Hank's balanced salt solution + 10% heat-inactivated fetal calf serum. The viability of cell suspensions was determined in a hemocytometer with trypan blue. Viability of cells derived from solid tumors ranged from 0–100% (median = 26%), with viability of cells from effusions and bone marrows ranged from 0–100% (median = 90%).

Culture Assay for Tumor Colony-forming Cells. Cells were cultured as described by Hamburger and Salmon [3, 4]. Cells to be tested were suspended in 0.3% agar in enriched CMRL 1066 medium (Grand Island Biological Company) supplemented with 15% horse serum, penicillin (100 units/ml), streptomycin (2 mg/ml), glutamine (2 m*M*), $CaCl_2$, (4 m*M*), and insulin (3 units/ml). Prior to plating, asparagine (0.6 mg/ml), DEAE-dextran (0.5 mg/ml; Pharmacia Fine Chemicals, Inc., Piscataway, NJ), and freshly prepared 2-mercaptoethanol (final concentration 50 μm) were added to the cells. One milliliter of the resultant mixture was pipetted onto 1-ml feeder layers in 35-mm plastic petri dishes. The final concentration of cells in each culture was 5×10^5 viable cells in 1 ml agar medium. The feeder layers used in this study consisted

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of McCoy's medium 5A plus 15% heat-inactivated fetal calf serum and a variety of nutrients [3, 4]. Immediately before use, 10 ml 3% tryptic soy broth (Grand Island Biological Company), 0.6 ml asparagine, and 0.3 ml DEAE-dextran were added to 40 ml enriched medium. Agar (0.5% final concentration) was added to the enriched medium and underlayers were poured in 35-mm petri dishes.

After preparation of both bottom and top layers, the plates were examined under an inverted microscope to assure the presence of a good single cell suspension. The plates were then incubated at 37° C in a 7% CO_2 humidified atmosphere.

Drug Sensitivity Studies. Stock solutions of standard anticancer agents were prepared in sterile buffered saline or water and stored at −70° C in aliquots sufficient for one assay. Tumor cell suspensions were transferred to tubes and adjusted to a final concentration of 1.0×10^6 cells/ml with the appropriate drug dilution or control medium. In an attempt to perform more drug studies per tumor specimen only one drug concentration was utilized. This single concentration corresponded to 1/10 the peak plasma concentration for each drug in man. Cells were incubated with and without drugs for 1 h at 37° C in Hank's balanced salt solution. The cells were then centrifuged at 150 *g* for 10 min, washed twice with Hank's balanced salt solution, and prepared for culture as described above.

After 14 days in culture the number of colonies on the triplicate control plates and triplicate drug-treated plates were counted and the percent decrease in colonies determined. Based on a previously reported retrospective clinical correlation study [13], a ≥ 70% decrease in colony number on drug-treated plates compared with control plates was set as a definition of sensitive in vitro. Thus for this study a ≥ 70% decrease in colony number in drug-treated plates versus control plates was defined as an in vitro response.

Scoring and Identification of Colonies in Cultures. Cultures were examined with a Zeiss inverted-phase microscope at × 30, × 100, and × 300. Colony counts were made 7, 14, and 21 days after plating. Aggregates of 50 or more cells were considered colonies and aggregates of less than 50 cells were considered clusters.

Results

Overall Growth Rates

Over the past 15 months a total of 2,365 patients' tumors have been placed in the soft agar culture system. Of these, 1,844 (78%) formed more than five colonies per 500,000 nucleated cell plated. This was defined as tumor growth in vitro. Of the 2,365 tumors plated, 1,206 (51%) formed ≥ 30 colonies per 500,000 cells plated and 499 (19%) of the 2,365 tumors plated formed ≥ 100 colonies per 500,000 nucleated cells plated.

Table 1 details the growth rate (≥ 5 colonies per 500,000 cells plated) for the major types of tumors placed in culture. Table 2 details the growth rate by the source of the clinical specimen. It is clear from these tables that a variety of tumors will form colonies in soft agar in a respectable percentage of culture attempts. In addition, it is clear that a higher percentage of malignant effusions and bone marrows

Table 1. Growth of tumor colonies from various human neoplasms

Type of Tumor	Number of patients with + culture/total tested	% Growth ≥ 5 colonies/plate	% Growth ≥ 30 colonies/plate
Breast	160/225	71	51
Ovarian	85/110	77	72
Melanoma	48/63	76	58
Colorectal	76/100	76	57
Neuroblastoma	72/80	90	80
Lung cancer			
Small	44/56	79	59
Squamous	48/52	92	72
Adenocarcinoma	44/48	92	76
Head and neck	28/64	44	27
Renal	29/31	94	82
Bladder	21/29	72	68
Testicular	17/24	71	48
Prostate	12/16	75	32
Stomach	9/15	69	45
Pancreas	11/14	79	58
Multiple myeloma	6/20	30	20
Rhabdomyosarcoma	6/12	50	33
Ewing's sarcoma	8/12	67	33
Osteogenic sarcoma	8/10	80	40
Hepatoma	6/6	100	67
Esophagus	2/3	66	33

containing tumors form colonies in soft agar than do solid tumor specimens. Table 3 details the mean, median, and range of colonies for each of the types of tumors which have formed colonies in the soft agar system. Average colony counts per 500,000 nucleated cells plated have ranged from 43–276, while median colony counts have ranged from 28–104 colonies per 500,000 cells plated.

Proof that the colonies growing in culture are indeed malignant has been reported elsewhere and will not be repeated here [3–7, 11, 12, 14, 17]. This proof has consisted in light microscopic and electron microscopy of cells growing in the colonies, secretion of tumor markers by the cells in the colonies, karyology, and plucking of colonies from soft agar with injection into nude mice and subsequent tumor formation at the site of inoculation in the mouse. All these studies have offered confirmational evidence that the colonies are indeed composed of tumor cells which are similar to the tumor growing in the patient.

Table 4 represents a summary of studies on various methods of specimen storage. In that table, storage of effusions at room temperature, 4° C, and 37° C has been compared over 24, 48, and 72 h. It is of note that malignant effusions can be stored at room temperature for as long as 72 h (as they are taken from the patient). Thus effusions can be stored or travel at room temperature for at least 3 days. These data agree with our previously reported experience with shipping and storage of bone marrows containing human neuroblastoma [17].

Initial experience with specimen cryopreservation utilizing 10% DMSO in McCoy's 5A has shown that there is a definite decrease in the number of colonies per number of viable cells plated (see Table 5). It is of interest, however, that the drug sensitivity profiles for fresh versus cryopreserved human tumor specimens

Table 2. Growth rate of malignancies in soft agar categorized by source of specimens

Source of specimen	Number of patients with colony growth/total tested	% Growth ≥ 5 colonies/plate	% Growth ≥ 30 colonies/plate
Solid tumor	424/801	53	41
Ascites	441/622	71	58
Bone marrow	357/401	89	76
Lymph node	142/222	64	52
Pleural effusion	246/311	79	68
Pericardial effusion	7/8	88	88

Table 3. Number of colonies formed by a variety of tumors growing in soft agar

Tumor type	No. of specimens which formed colonies	Mean colony count	Median colony count	Range of colony count
Breast	160	117	45	4–1752
Ovarian	85	216	54	7–1680
Melanoma	48	187	66	6–2930
Colorectal	76	125	51	7–1066
Neuroblastoma	72	276	38	6–7214
Lung cancer				
Small	44	130	41	12– 848
Squamous	48	155	65	19– 674
Adenocarcinoma	44	196	80	4–1465
Head and neck	28	111	28	5–1152
Renal	29	211	81	11–1321
Bladder	21	92	104	5– 178
Testicular	17	73	38	8– 251
Prostate	12	133	58	14– 610
Stomach	9	100	49	9– 537
Pancreas	11	72	42	6– 619
Multiple myeloma	6	183	24	6–1233
Hepatoma	6	172	30	12–2396
Esophagus	2	43	42	14– 83

Table 4. Effect of storage temperature and length of storage on viability and growth of tumors from malignant effusions

Tumor type	Control (1 h)[a]			24 h			48 h			72 h		
	20° C	4° C	37° C	20° C	4° C	37° C	20° C	4° C	37° C	20° C	4° C	37° C
Breast	118[b]	110	50	110	62	31	100	52	11	111	82	40
Breast	78	52	20	88	50	11	70	37	10	68	31	0
Breast	215	106	42	211	118	0	226	42	6	198	30	0
Breast	64	28	31	58	20	0	51	10	0	40	11	0
Ovarian	512	418	118	480	398	101	462	369		318	271	87
Ovarian	132	129	47	148	111	36	107	92	0	84	63	0
Ovarian	61	49	22	57	58	0	42	42	0	47	31	0
Ovarian	37	31	18	41	36	7	18	0	0	9	0	0
Colon	32	21	12	36	0	0	31	0	0	29	0	0
Colon	81	62	19	72	0	0	61	0	0	64	0	0
Colon	62	58	4	50	32	0	41	18	0	58	11	0
Colon	37	22	0	28	0	0	18	6	0	11	0	0
Hepatoma	68	51	18	72	22	11	84	31	0	61	16	0

[a] Controls represent cells that were stored for **1 h**, then washed and resuspended in CMRL 1066 + 10% FCS
[b] Number of colonies per 500,000 cells plated

Table 5. Drug sensitivity profile of fresh versus cryopreserved human tumor cells growing in soft agar

Tumor type	Fresh	Cryopreserved	No. of colonies control plates	Drug	Concentration μg/ml	% Decrease in tumor colony forming units (TCFU's)
Ovarian	×		304	ADRIA	0.1	24
Ovarian		×	201	ADRIA	0.1	31
Ovarian	×		42	M-AMSA	1.0	71
Ovarian		×	24	m-AMSA	1.0	54
Pancreas	×		119	DHAD[a]	0.1	18
Pancreas		×	82	DHAD[a]	0.1	27
Small cell	×		181	ADRIA	0.1	71
Small cell		×	98	ADRIA	0.1	68
Colorectal	×		78	5FU	10	51
Colorectal		×	42	5FU	10	41
Breast	×		318	ADRIA	0.1	71
Breast		×	111	ADRIA	0.1	58

[a] Dihydroxyanthracenedione or Mitoxantrone

remains qualitatively the same (see Table 5). More experimentation in this area is clearly required.

The methods for storing solid tumors or single cell suspensions from these tumors are currently being investigated. At present, however, these specimens require processing within 6 h of receipt of the specimen.

In vitro-in vivo Correlations for Individual Patient Drug Sensitivities

After the initial reports by Salmon and colleagues detailing utilization of the soft agar technique for determining chemosensitivity of an individual patient's tumor to a particular drug [8, 9], we embarked on a retrospective trial comparing in vitro cell kill in soft agar with clinical response of a patient's tumor to the same drug. These results have been reported in more detail elsewhere [13]. In brief, 800 patients' tumors were cultured. Of these 800 tumors, 459 (57%) grew (formed ≥ 5 colonies per 500,000 cells plated). The number of specimens which grew ≥ 30 colonies per plate and had enough cells for at least one drug assay was 199, or 25% of the patients' tumors we started with. Thus the first important finding has been that with the present methodology, in vitro drug sensitivity can only be determined in 25% of a general oncology patient population.

Table 6. In vitro/in vivo associations

No. of patients	Sensitive in vitro and in vivo	Sensitive in vitro/ resistant in vivo[a]	Resistant in vitro/ sensitive in vivo[b]	Resistant in vitro and in vivo	Total number of correlations
101	15	6	2	100	123

[a] False positives
[b] False negatives

Table 7. Clinical versus in vitro (cloning system) responses to adriamycin

Tumor type	% Response rate in man[a]	% Response rate in cloning assay[b]
Breast	37	22
Lung		
Small cell	33	28
Adenocarcinoma	21	10
Ovarian	16	12
Neuroblastoma	30	12
Prostate	14	20
Bladder	22	20
Leukemia (AML)	39	40
Lymphoma	32	20
Sarcoma	27	17
Colorectal	13	0
Melanoma	0	0

[a] From reference [1]
[b] Response defined as $\geq 70\%$ decrease in tumor colony forming units in vitro

For the 199 patients' tumors that did have adequate cells for drug testing and adequate growth in soft agar, we were able to perform 904 drug sensitivity tests. The single drug concentrations utilized in the study are detailed elsewhere [13]. Because this was a retrospective study, only 123 of those 904 drug tests were actually performed in 101 patients. One half of the patients had been refractory to prior chemotherapy. Table 6 shows the retrospective in vitro-in vivo associations. It is of note that there were 16 instances in which an in vitro response correctly corresponded to a clinical complete or partial response (true-positive). There were six false-positives for the assay, two false-negatives and 100 true-negatives.

Overall, the probability of a positive association from the assay, given the patient responded in vivo (sensitivity), was 0.88, while the probability of a positive association, given the patient did not respond (specificity), was 0.06. Thus the probability of a negative association, given the patient did not respond, was 0.94. Associations between in vitro and in vivo results in the entire 101 patients were highly significant ($P < 0.001$) [13].

It should be emphasized that these results are from a retrospective trial. However, they are interesting enough to warrant a prospective clinical trial for the assay.

Screening of New and Old Drugs

Workers in our laboratory and others have suggested that the human tumor cloning system may be utilized to screen for in vitro activity of a number of new or old antineoplastics [10, 14, 15, 18]. Use of the system for an in vitro phase II trial could help pinpoint where a new agent has phase II activity. The first phase II clinical trials could then concentrate on the tumor types where the drug has in vitro activity. This methodology could conceivably save patients, time, and money.

To determine whether the assay could be used to screen for new agents it would be desirable to determine whether some of the known clinically active drugs would appear as active in the human tumor cloning assay. Adriamycin was tested in vitro against 383 patients' tumors. None of these patients had had any prior chemotherapy. As expected, only 226 (59%) of the tumors grew ≥ 30 colonies in control plates and thus were evaluable for drug sensitivity studies. The in vitro response rates for these 226 tumors are noted in Table 7. It is encouraging that for the most part the in vitro results are very similar to results obtained in the clinical trials. These data support the concept that the cloning assay would pick up a new agent which is active in a variety of tumor types.

We have screened a large number of both old and investigational agents in the cloning system. Table 8 details the in vitro response rates of 186 evaluable tumors to MGBG (methylglyoxalbisguanhydrazone). The drug appears to be inactive against colorectal cancer, ovarian cancer, and hypernephroma. The drug does deserve clinical testing against pancreatic and bladder cancer, hepatoma, and adenocarcinoma and large cell carcinoma of the lung.

Table 8. In vitro antitumor activity of MGBG

Tumor type	No. of responses[a]/ no. evaluable	% Response rate
Colorectal	3/35	9
Hypernephroma	1/33	3
Ovarian	0/13	0
Lung, squamous	0/10	0
Melanoma	0/9	0
Pancreas	2/9	22
Bladder	1/8	13
Lung adeno	1/7	14
Hepatoma	1/6	17
Lung, large cell	1/3	33

[a] In vitro response = $\geq$ 70% kill

Monitoring of Bone Marrow for Tumor Involvement

Our laboratory has reported on use of the human tumor cloning system to monitor bone marrow for involvement by tumor [7, 17]. In the first blind study, bone marrows with and without neuroblastoma were sent from Milwaukee Children's Hospital, Medical College of Wisconsin, to the cloning laboratory in San Antonio, Texas. There were 38 instances in which histologic study of the specimen demonstrated neuroblastoma cells. The soft agar system showed colony growth in 30 of the 38 samples (79%). There were a total of 38 specimens which were histologically negative for neuroblastoma. Of these 38 specimens, 30 showed no growth in the cloning assay. Eight histologically negative specimens from six patients formed colonies in the soft agar system. Five of these six patients showed tumor histologically on prior or subsequent marrow examinations. There was a highly significant correlation between histologic and soft agar culture results ($P < 0.001$) [17]. The ability of the assay to document marrow involvement by small cell lung cancer has also been proven [7]. This finding could enable the cloning assay to be used to monitor a patient's bone marrow for tumor involvement before the marrow is harvested for autologous bone marrow re-infusion. This monitoring could help avoid re-infusing tumor-containing bone marrow.

Use of Cloning System as a Prognostic Factor

It is clear from Table 3 that patients' tumors vary in their ability to form colonies in soft agar. Some tumors form many colonies, while some form very few. This raises the question as to whether tumors which grow more rapidly in soft agar might be 'more aggressive' tumors. To study this, the cloning efficiency (no. of colonies grown/500,000 cells plated $\times$ 100) was evaluated as a prognostic factor for survival. For 27 head and neck cancer patients we have determined that those with a cloning efficiency of $> 0.02\%$ have a statistically significantly shorter survival than those with a cloning efficiency of $< 0.02\%$ [6]. This inverse relationship between cloning efficiency and survival also holds for neuroblastoma, the only other tumor studied [17]. Whether the inverse relationship between cloning efficiency and survival is an epiphenomenon for all tumor types is as yet unknown.

Discussion

The first conclusion from this large experience with the human tumor cloning system is that a large number of the histologic types of malignancies will form colonies in soft agar. However, only 51% of the 2,365 specimens plated formed $\geq$ 30 colonies. At least 30 colonies are needed in control plates to assure an adequate baseline for drug sensitivity testing. Future research efforts must be directed toward increasing the growth rate and, if possible, the number of colonies per number of cells plated. Improving methods of storage, transport, and cryopreservation as outlined in this study is a step in that direction. Exploration of growth factors is also a promising area.

Despite the fact that not all tumors will grow and not all will form enough colonies in vitro, there are a number of clinical correlation studies that can be performed. As noted above in retrospective trials that there have been excellent associations between in vitro drug sensitivity results and results noted with the same drug used in the clinic. Our results are very similar to the results reported by Salmon and colleagues [8, 9]. It is clear, however, that before there is widespread use of the system for predicting a patient's response to chemotherapy that prospective trials of the system are an absolute requirement.

The assay clearly has a place in screening for new anticancer agents. We have shown that the assay would pick up the commonly used antineoplastics. The assay has already been used to try to predict where the new antitumor agent, Mitoxantrone, might have phase II activity in clinical use [18]. If the system is predictive for phase II activity, it would allow pinpointing of the tumor types where the drug should be tried first. This would lead to considerable savings in patients and time.

Use of the assay system for monitoring patients' bone marrow for tumor involvement has now been demonstrated. This clinical application could be

incorporated into initial staging procedures for a variety of malignancies (e.g., for small cell lung cancer, marrow involvement by tumor denotes extensive disease). In addition, the assay system could be used to monitor bone marrows for tumor involvement in autologous bone marrow transplantation programs.

Finally, we have demonstrated that the number of colonies growing in the cloning assay may be used as a prognostic factor for survival. This finding has several applications, including predicting patient survival and possibly in the future even determining which patients might require adjuvant treatment with chemotherapy, as opposed to watchful waiting.

In summary, while the cloning assay does have several practical problems, it is an entirely feasible system which can be used to study a number of clinical problems.

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References

1. Blum R, Carter SK (1974) Adriamycin: A new antitumor drug with significant clinical activity. Ann Int Med 80: 249
2. Bradley EC, Reichert CM, Brennan MF, Von Hoff DD (1980) Direct cloning of human parathyroid hyperplasia cells in soft agar culture. Cancer Res 40: 3694
3. Hamburger A, Salmon SE (1970) Primary bioassay of human myeloma stem cells. J Clin Invest 60: 846
4. Hamburger A, Salmon SE (1977) Primary bioassay of human tumor stem cells. Science 197: 461
5. Hamburger AW, Salmon SE, Kim MB, Trent JM, Soehnlen BJ, Alberts DS, Schmidt HJ (1978) Direct cloning of human ovarian carcinoma in agar. Cancer Res 35: 3438
6. Mattox DE, Von Hoff DD (1980) In vitro stem cell assay in head and neck squamous carcinoma. Ann J Surg 140: 527
7. Pollard EB, Tio F, Whitecar JP, Coltman CA Jr, Von Hoff DD (1980) Utilization of a soft agar system to monitor for marrow involvement with small cell carcinoma of the lung. Proc Am Assoc Cancer Res 21: 192
8. Salmon SE, Soehnlen BJ, Durie BGM, Alberts DS, Meyskens FC, Chen HSG, Moon TE (1979) Clinical correlations of drug sensitivity in tumor stem cell assay. Proc Am Assoc Cancer Res 20: 340
9. Salmon SE, Hamburger AW, Soehnlen B, Durie BGM, Alberts DS, Moon TE (1980a) Quantitation of differential sensitivity of human tumor stem cells to anticancer agents. N Engl J Med 298: 1321
10. Salmon SE, Alberts D, Meyskens F, Durie B, Jones S, Soehnlen B, Young L (1980b) A new concept: In vitro trial with the human tumor cell assay (HTSCA). Proc Am Assoc Cancer Res/ASCO 21: 329
11. Sandback J, O'Brien M, Welch D, Cohen SC, Gorden DH, Rodriguez V, Whitecar JP Jr, Von Hoff D (1980) Assay for clonogenic cells in human breast cancer. Proc Am Assoc Cancer Res/ASCO 21: 139
12. Sarosdy M, Von Hoff DD, Lamm D, Radwin H (1981) Clonogenic assay and in vitro chemosensitivity testing of human urologic malignancies. Cancer (in press)
13. Von Hoff DD, Casper J, Bradley E, Sandbach J, Jones D, Makuch R (1981) Association between human tumor colony forming assay results and response of an individual patient's tumor to chemotherapy. Am J Med 10: 1027
14. Von Hoff DD (1980b) New leads from the laboratory for treating testicular cancer. In: Theapeutic progress in ovarian cancer, testicular cancer and sarcomas. Martin Nijhoff, The Hague Boston London, p 225
15. Von Hoff DD, Bradley F (1979) Use of a human tumor stem cell assay system to screen for phase II activity of new antineoplastic agents. Proceedings of the IX International Conference on Antimicrobials and Chemotherapy. Abstract no. 920
16. Von Hoff DD, Johnson GE (1979) Secretion of tumor marker in the human tumor stem cell system. Proc Am Assoc Cancer Res 20: 51
17. Von Hoff DD, Casper J, Bradley E, Trent JM, Hodach A, Reichert C, Makuch R, Altman A (1980a) Direct cloning of human neuroblastoma cells in soft agar culture. Cancer Res 40: 3591
18. Von Hoff DD, Coltman CA Jr, Forseth B, Murray F (1981b) Activity of Mitoxantrone in a human tumor cloning system: Leads for phase II trials in man. Cancer Res 41: 1853

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