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Feasibility of Drug Screening with Panels of Human Tumor Cell Lines Using a Microculture Tetrazolium Assay[1]

Michael C. Alley,[2] Dominic A. Scudiero, Anne Monks, Miriam L. Hursey, Maciej J. Czerwinski, Donald L. Fine, Betty J. Abbott, Joseph G. Mayo, Robert H. Shoemaker, and Michael R. Boyd

Program Resources, Inc., National Cancer Institute-Frederick Cancer Research Facility, Frederick, Maryland 21701 [M. C. A., D. A. S., A. M., M. L. H., M. J. C., D. L. F.] and Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute, Bethesda, Maryland 20892 [B. J. A., J. G. M., R. H. S., M. R. B.]

ABSTRACT

For the past 30 years strategies for the preclinical discovery and development of potential anticancer agents have been based largely upon the testing of agents in mice bearing transplantable leukemias and solid tumors derived from a limited number of murine as well as human sources. The feasibility of implementing an alternate approach, namely combined *in vitro/in vivo* screening for selective cytotoxicity among panels of human tumor cell lines derived from a broad spectrum of human solid tumors is under investigation. A group of 30 cell lines acquired from a variety of sources and representing 8 lung cancer pathologies as well as 76 cell lines representing 10 other categories of human cancer (carcinomas of colon, breast, kidney, prostate, ovary, head and neck; glioma; leukemia; melanoma; and sarcoma) have exhibited acceptable growth characteristics and suitable colorimetric profiles in a single, standard culture medium. Measurements of *in vitro* growth in microculture wells by cell-mediated reduction of tetrazolium showed excellent correlation ($0.89 < r^2 < 0.98$) with measurements of cellular protein in adherent cell line cultures as well as viable cell count in suspension cell line cultures ($0.94 < r^2 < 0.99$). Since the microculture tetrazolium assay provides sensitive and reproducible indices of growth as well as drug sensitivity in individual cell lines over the course of multiple passages and several months' cultivation, it appears suitable for initial-stage *in vitro* drug screening.

INTRODUCTION

A new anticancer drug screening program based upon the use of multiple panels of human solid tumor cell lines is under development by the U. S. National Cancer Institute's Developmental Therapeutics Program, Division of Cancer Treatment (1–7). The goal of the new program is to evaluate experimental agents against groups of cell line panels each representing a major clinical category of human malignancy. Each panel (*e.g.*, lung, colon, melanoma, renal, ovarian, and central nervous system) is to contain multiple, representative human tumor cell lines. Agents showing differential or selective patterns of *in vitro* growth inhibition will be evaluated subsequently in athymic mice bearing the same human tumor cell lines found sensitive *in vitro*. This *in vitro/in vivo* approach differs from previous *in vivo* screening programs (8, 9), which most recently consisted of a murine leukemia prescreen followed by a battery of tests including several murine tumor models and three human tumor xenografts, in two fundamental ways: (*a*) a single *in vivo* murine leukemia prescreen step replaced by broad-based *in vitro* evaluations among a wide variety of cell lines; and (*b*) the major clinical forms of human solid tumors represented by panels of multiple well-characterized human tumor cell lines. In addition, the proposed *in vitro* component differs from other *in vitro* screening methodologies, *e.g.*, the human tumor colony formation assay (10, 11) which is limited by its labor-intensive nature and the more limited range of tumor types amenable to soft-agar culture.

To determine whether such an *in vitro/in vivo* disease-oriented screening program is feasible, we have examined a number of technical questions which are fundamental to *in vitro* assay systems. In this report we have assessed whether it is possible to cultivate a multiplicity of human tumor cell lines under similar culture and assay conditions; whether a colorimetric assay (*e.g.*, 12, 13) is suitable for the measurement of cell line growth inhibition; and whether individual cell lines exhibit stable and reproducible drug sensitivity profiles over time.

MATERIALS AND METHODS

Cell Line Expansion, Cryopreservation, and Characterization

Thus far, more than 100 tumor cell lines representing many human solid tumor types have been acquired from several sources following various methods of isolation and cultivation. Individual cell lines were initially photographed, expanded (two passages maximum) and cryopreserved (*master stocks*) with growth medium and split-ratios recommended by their respective sources. Only cell lines documented to be free of adventitious bacteria and pathogenic viruses (NCI-FCRF[3] Diagnostic Microbiology Lab and Animal Health Diagnostic Lab) were accepted for subsequent characterization. Following recovery of master stocks, cell lines were adapted to a single, standard culture medium: RPMI 1640 (Quality Biologicals, Inc., Gaithersburg, MD) supplemented with 10% heat-inactivated fetal bovine serum (Sterile Systems Hyclone, Logan, UT) and 2 mM L-glutamine (NCI-FCRF Central Medium Laboratory) without antibiotics and cultured under conventional culture conditions, that is, 37°C, 5% CO_2, 95% air, 100% relative humidity. Cell lines were then expanded (five passages maximum) and cryopreserved for generation of seed stocks. Established adherent cell monolayers approaching 80% confluency were harvested with trypsin/EDTA (NCI-FCRF Central Medium Laboratory) whereas some early passage adherent cell lines were harvested with solution A and 2×-crystalized trypsin III (Sigma Chemical Co.) according to the protocol of Shipley and Ham (14). Leukemia cell lines were subcultured by trituration and dilution. Small cell lung carcinoma cell lines (which generally form large aggregates in suspension under conventional culture conditions) were cultured and assayed in suspension as well as adherent monolayers utilizing poly-L-lysine pretreatment of culture vessels (15). Following recovery of seed stock, cell lines were subjected to isoenzyme analysis as well as preliminary growth and drug sensitivity assays using one or more *in vitro* growth inhibition assays (described below). Cell lines meeting basic quality assurance criteria (mycoplasma-

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[2] To whom requests for reprints should be addressed at: PDRG, Developmental Therapeutics Program, National Cancer Institute-Frederick Cancer Research Facility, Building 560, Room 32-60, Frederick, MD 21701.

[3] The abbreviations used are: NCI-FCRF, National Cancer Institute-Frederick Cancer Research Facility; DMSO, dimethyl sulfoxide; INT, 2-(*p*-iodonitrophenyl)-3-*p*-nitrophenyl-5-phenyl tetrazolium chloride; MCPA, microculture cellular protein assay; MTA, microculture tetrazolium assay; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; NBT, 2,2′-di-*p*-nitrophenyl-5,5′-diphenyl-3,3′-(3,3′-dimethoxy-4,4′-diphenylene)ditetrazolium chloride; IC_{50}, 50% of control growth absorbance.

negative, MAP-negative, human isoenzymes only) and exhibiting suitable growth profiles were expanded (five serial passages from seed stock, maximum) and cryopreserved as a large number of aliquots designated working seed stock. Cell cryopreservation was achieved using a CryoMed controller (Model 801) and a CryoMed freezing chamber (No. 2700) with a step rate of −1°C/min followed by storage in vapor-phase liquid N_2 (NCI-FCRF Central Repository). Cell line seed stocks were tested also for *in vivo* tumorigenicity (s.c. and i.p. inoculations) in accordance with established protocols (16). Cell lines recovered from working seed stocks were subjected to repeat mycoplasma tests and to more extensive *in vitro* growth characterization. Cell lines subsequently were evaluated with respect to stability in drug sensitivity profiles over the course of 20 weekly passages. In addition, each cell line was expanded (eight passages, maximum from seed stock thaw) and cryopreserved as a large number of aliquots ("roller bottle" stock) for *in vivo* characterization and assay development.

Reagents

Tetrazolium/formazan reagents were purchased from Sigma Chemical Co. (St. Louis, MO): MTT (M2128), MTT formazan (M2003), INT (I8377), INT formazan (I7375), and NBT (N6876). DMSO was purchased from Sigma Chemical Co. (D5879), J. T. Baker Chemical Co. (9194-3, Phillipsburg, NJ), and American Burdick and Jackson Laboratories (Spectrophotometric Grade Product 081, Muskegan, MI). These chemicals were stored in unopened bottles at room temperature in the dark or in 50-ml sterile plastic tubes at −20°C in the dark. Anhydrous isopropanol (505-7) and propylene glycol (P-1009) were purchased from Sigma Chemical Co. Reagent grade hydrochloric acid (A-744) and hexane (H-302-1) were purchased from Fisher Scientific Co. Dimethylformamide (27,054-7) was purchased from Aldrich Chemical Co. (Milwaukee, WI) Propanol (spectrophotometric grade 9068-1) was purchased from American Burdick and Jackson Laboratories.

All chemotherapeutic agents were obtained from the Drug Synthesis and Chemistry Branch, Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute. Crystalline stock materials were stored at −20°C. Solvent-reconstituted chemotherapeutic agents were prepared at high concentration, partitioned into multiple aliquots, and stored at −70°C. Just prior to culture application the contents of frozen vials were thawed and mixed. Measured aliquots (20–200 μl) were transferred by micropipet (Gilson Pipetman, Models P200 and P1000) equipped with polypropylene tips to standard culture medium within polypropylene tubes (Sarstedt 62.554/002 or Falcon 2098) and serially diluted in culture medium containing an appropriate concentration of vehicle.

In Vitro Growth/Growth Inhibition Assays

Microculture Tetrazolium Assay. The methodology described below represents a modification of the original MTT colorimetric assay described by Mosmann (12): In principle, the viable cell number/well is directly proportional to the production of formazan, which following solubilization, can be measured spectrophotometrically. Our modification of the original *in vitro* assay procedures has been previously described (6, 17). In brief, cells were harvested from exponential-phase maintenance cultures (T-75 cm^2 flasks; Falcon Plastics 3023), counted by trypan blue exclusion, and dispensed within replicate 96-well culture plates (Falcon Plastics 3075) in 100-μl volumes using a repeating pipet (Eppendorf repeater 4780) or multichannel pipet (Flow Labs, Titertek). Following a 24-h incubation at 37°C, 5% CO_2, 100% relative humidity (Heraeus B5060EKO_2 incubators or NAPCO 5300 incubators), 100 μl of culture medium, culture medium containing drug or culture medium containing drug vehicle was dispensed within appropriate wells (vehicle control group, $N = 6$; each drug treatment group, $N = 3$). Peripheral wells of each plate (lacking cells) were utilized for drug blank ($N = 2$) and medium/tetrazolium reagent blank ($N = 6$) "background" determinations. Culture plates were then incubated for 1 to 11 days prior to the addition of tetrazolium reagent. MTT stock solution was prepared as follows: 5 mg MTT/ml PBS (Quality Biologicals, Inc.) was sterile filtered with 0.45-μm filter units (Nalgene type SCN) and stored at 4°C for a maximum of 1 month. MTT working solution was prepared just prior to culture application by diluting MTT stock solution 1:5 (v/v) in prewarmed standard culture medium. Alternatively, other tetrazolium reagents (namely, INT or NBT) were prepared and utilized in a similar fashion for selected experiments. Under standard MTA conditions 50 μl of MTT working solution was added to each culture well (resulting in 50 μg MTT/250 μl total medium volume) and cultures were incubated at 37°C for 4 to 24 h depending upon individual cell line requirements. Following incubation cell monolayers and formazan were inspected microscopically: Culture plates containing suspension lines or any detached cells were centrifuged at low speed for 5 min. All but 10–20 μl of culture medium supernatant was removed from wells by slow aspiration through a blunt 18-gauge needle and replaced with 150 μl of DMSO (Burdick & Jackson) using a multichannel pipet. Following thorough formazan solubilization (trituration by pipet or vibration on a plate shaker), the absorbance of each well was measured using a microculture plate reader (Dynatech MR600; Alexandria, VA) at 540 nm (single wavelength, calibration factor = 1.00) interfaced with an Apple IIe computer. Subsequently, data were stored and analyzed through use of Apple Soft, Apple Turnover, and Lotus Symphony software. Cell line growth and growth inhibition were expressed in terms of mean (±1 SD) absorbance units and/or percentage of control absorbance (±1 SD%) following subtraction of mean "background" absorbance. Linearity and reproducibility of instrument measurements were verified by the use of formazan reagents in appropriate solvent systems.

Absorption spectra of formazan reagents as well as cell-generated formazans were measured with a UV/visible scanning spectrophotometer (Perkin-Elmer Lambda V; Perkin-Elmer Corp., Norwalk, CT). Samples were placed in 1-cm pathlength disposable polystyrene cuvets (Fisher Scientific Co. 14-385-942) except those solubilized in dimethylformamide solvent which were evaluated in 1-cm pathlength glass spectrophotometer cells (Coleman S7300-4). Freshly prepared material was analyzed in dual beam mode with 2-nm slit width, at 120 nm/min, 0.02 A threshold, and 0.5 s response. Instrument wavelength calibration was verified by examination of deuterium emission spectra to be 653.1 ± 0.3 nm.

Microculture Cellular Protein Assay. A cellular protein assay previously described by Finlay, Baguley, and Wilson (13) was adapted to the measurement of cell line growth under the same culture conditions described above for the MTA. Following 1–11 days' incubation, supernatant culture medium was removed and 200 μl of methylene blue (Sigma MB-1) solution [5 g/liter in ethanol:water (50%, v/v)] was added without delay. Following incubation at room temperature for 45 min, unbound stain was removed by plate inversion on absorbant paper and subsequent emersion/dilution in four, 1-liter washes with distilled deionized water. Bound protein stain was solubilized by the addition of 100 μl SDS (Sigma L4509) solution (1%, v/v in water) to each well. Absorbances of wells were measured at 630 nm (single wavelength, calibration factor = 1.00) using equipment and computerized analysis procedures described above for the MTA.

RESULTS

Cell Line Acquisition, Adaptation, and Cryopreservation. A key question concerning the use of *in vitro* cell lines for comparative drug evaluation has been whether a wide variety of cell lines would exhibit stable growth and drug sensitivity profiles over serial passage. To examine this question at a practical level we have evaluated the performance of multiple cell lines which represent common human solid tumor malignancies.

To date 111 cell lines derived from 10 major categories of human cancer (carcinomas of lung, colon, kidney, ovary, prostate, and head and neck; glioma; leukemia; melanoma; and sarcoma) isolated by a variety of *in vivo* as well as *in vitro* techniques have been acquired, cultivated, cryopreserved, and tested. With the exception of two early passage colon adenocarcinoma lines and one lung adenosquamous line which exhibit doubling times exceeding 120 h in our standard culture medium

formulation (each of which also proliferates slowly in their respective recommended culture medium) all tumor cell lines tested to date show suitable growth under maintenance (T-75 cm^2 flask) as well as assay conditions (96-well plate). In addition, two fibroblast cell lines (which exhibit acceptable rates of growth) metabolized MTT at levels which are adequate for detection but which may not be desirable for screening (<0.500 absorbance units/confluent monolayer). A total of 106 cell lines which meet basic quality assurance criteria and which exhibit suitable growth and colorimetric profiles are listed in Table 1. Despite the fact that these cell lines have been cultivated under a variety of conditions (including 31 different culture medium formulations) in other laboratories, each cell line appears to have adapted adequately to one set of culture conditions (and a single, standard culture medium) as indicated by exponential or near-exponential growth following inoculation at reasonable cell densities (<10,000 cells/well).

Microculture Tetrazolium Assay. Preliminary experiments using the original MTT colorimetric assay (6) revealed an apparently inadequate level of formazan generation by some cell lines, limited solubility and stability of MTT formazan, and an incompatability of the acid/isopropanol solvent system with the evaluation of some synthetic agents. Microscopic inspection of plates following tetrazolium metabolism (prior to solvent addition) revealed that these problems generally were not due to the ability of cells to metabolize MTT. Some cell lines such as NCI-H460 produced copious amounts of formazan much of which was insoluble in acid/isopropanol; other cell lines such as NCI-H322 and P388 exhibited significant formazan microscopically but low absorbance readings, again due to limited solubility of cell-generated formazan.

These findings prompted assessment of other formazan solvent systems. Solubility testing and spectral analysis with a number of solvents including DMF, DMSO, hexane, and propylene glycol showed that DMSO was the most suitable solvent for culture-generated MTT formazan as well as INT formazan; NBT formazan was not appreciably soluble in any of these neat solvents at room temperature. Microscopic inspection of cell culture plates revealed that formazans are rapidly mobilized by DMSO from sites within thick cell layers. Spectrophotometric analyzes indicate that the resulting DMSO/formazan solutions are stable and exhibit prominent absorbance in the visible light region (Fig. 1*B*). While MTT formazan reagent is in fact totally soluble in anhydrous isopropanol at a concentration of 5 mg/ml, in the presence of 0.04 N HCl/isopropanol as specified by the original procedure (12), MTT formazan exhibits a very blunted absorbance at 570 nm and increased absorbance at 420 and 300 nm (see Fig. 1*D*). Color "fading" was accompanied by a rapid, irreversible shift in absorbance maximum and appeared to be a direct consequence of medium acidification. The absorbance of MTT formazan reagent in DMSO is approximately 1.3× that of formazan in neat isopropanol (Fig. 1*C*) and more than 6.2× that observed in the acid/isopropanol solvent system. While the extinction coefficient of MTT formazan reagent in dimethylformamide (E_{513} = 18,100 M^{-1} cm^{-1}) was the highest of all neat solvents tested (Fig. 1*A* and Table 2), dimethylformamide is not compatible with polystyrene culture vessels. On the grounds of improved solubility and stability of culture-generated MTT formazan in DMSO, this solvent was adopted in our current microculture tetrazolium assay.

Spectral characteristics of reagent as well as culture-generated MTT formazan depend not only upon the organic solvent but also upon the presence or absence of serum. As shown in Fig. 2*A*, the absorption spectra of culture-derived MTT formazan and MTT formazan reagent differ significantly. The former exhibits an absorbance maximum of 553 nm (half-height bandwidth of 110 nm), whereas the later exhibits an absorbance maximum of 506 nm (half-height bandwidth of 170 nm). In the presence of DMSO and low serum concentration MTT formazan reagent exhibits a shift in the absorption maximum from 506 to 553 nm and a narrowing of the half-height bandwidth to 108 nm (Fig. 2*B*), a profile consistent with that of culture-derived MTT formazan.

As shown in Fig. 2*C* the presence of 0.5 to 5.0% (v/v) serum in DMSO substantially enhances (2.3× maximum) the molar extinction coefficient of MTT formazan (E_{506} = 15,400 *versus* E_{553} = 36,300 M^{-1} cm^{-1}). Serum concentrations of 0.5 to 5.0% in 150 μl DMSO (volume of solvent employed in final step of MTA) are equivalent to 31.5–315 μg protein/culture well (since the total protein concentration of the fetal bovine serum lot was 42 mg/ml). The presence of 10 μl (or somewhat more) culture medium (containing 10% FBS) which remains following the aspiration step coupled with protein adsorbed to culture well surfaces and cell monolayers provides an adequate amount of protein (more than 42 μg) for formazan complex formation and nearly maximum extinction. DMSO rapidly solubilizes serum as well as formazan, whereas isopropanol, propanol, hexane, and dimethylformamide are incapable of solubilizing serum at concentrations exceeding 0.0625% (data not shown). While serum is highly soluble in propylene glycol (>10% v/v is achievable), this solvent does not adequately mobilize cell-generated MTT formazan. The combined solubility of serum and formazan in DMSO unlike other organic solvents appears to be responsible for the improved extraction and detection of MTT formazan generated within cultured cell systems.

The effect of serum upon formazan extinction is not unique to MTT formazan. DMSO and serum over the same range of serum concentrations also enhance extinction and narrow the absorption bandwidth of INT formazan. While the molar extinction coefficients of INT formazan in DMSO and DMSO/serum exceeds that of MTT formazan (Table 2), the rate of formazan generation by cells is more rapid for MTT than for INT. In addition, MTT formazan (blue violet) is easier to visualize than INT formazan (red) in cell culture by light microscopy.

While MTT formazan reagent in DMSO exhibits stable spectrophotometric characteristics for several days, the absorbance of MTT formazan derived from cell culture (under conditions described for MTA above) begins to change within several hours and is affected by DMSO grade and stock DMSO storage conditions. Use of spectrophotometric grades of DMSO provide stable "background" absorbance levels for up to 2 h following solvent application, whereas use of nonspectrophotometric DMSO preparations or DMSO preexposed to air are accompanied by ever-increasing levels of "background" absorbance within 15 min of solvent application.

Microculture Growth Evaluations. *In vitro* growth evaluations on candidate cell lines were performed as follows. For each cell line a range of inoculation densities (40–20,000 cells/well) and culture durations (usually 1, 2, 4, 8, and 11 days) were evaluated (*e.g.*, Fig. 3, *A* and *B*). From such data it was possible to determine which inoculation densities give rise to a detectable and linear range of absorbance readings for a given culture duration.

To assess whether the absorbance arising from MTT formazan in adherent cell line cultures reflects total cell mass/well, replicate culture plates for each of four cell lines (A549, NCI-H460, NCI-H322, and NCI-H23) were subjected to the MTA

Table 1 *Established cell lines employed for initial in vitro growth evaluations and MTA development*

Histology cell line	Reference	Institution (Source[a])	Culture medium[b]	MTA inoculation density range[c]
Non-small cell lung cancer				
Adenocarcinoma				
A549	18	National Cancer Institute (ATCC)	5	78–1,250
A549/Asc-1	–	National Cancer Institute (NCI-TB)	17	312–2,500
Calu-3	19	Memorial Sloan-Kettering Cancer Center (ATCC)	3	156–5,000
Calu-6	19	Memorial Sloan-Kettering Cancer Center (ATCC)	3	156–2,500
EKVX	–	Norsk Hydro's Institute, Norway (O. Fodstad)	17	156–2,500
NCI-H23	20, 21	National Cancer Institute (A. F. Gazdar)	17	312–2,500
NCI-H324	22	National Cancer Institute (A. F. Gazdar)	17	156–5,000
NCI-H522	22	National Cancer Institute (A. F. Gazdar)	17	39–5,000
Adenosquamous carcinoma				
NCI-H125	21, 22	National Cancer Institute (A. F. Gazdar)	17	312–5,000
NCI-H647	–	National Cancer Institute (A. F. Gazdar)	17	156–5,000
Squamous cell carcinoma				
NCI-H520	21–23	National Cancer Institute (A. F. Gazdar)	17	78–5,000
NCI-H226	21, 23	National Cancer Institute (A. F. Gazdar)	17	312–2,500
SK-MES-1	19	Memorial Sloan-Kettering Cancer Center (ATCC)	3	39–1,250
Bronchiolo-alveolar carcinoma				
NCI-H322	21, 24	National Cancer Institute (A. F. Gazdar)	17	156–5,000
NCI-H358	21–24	National Cancer Institute (A. F. Gazdar)	17	156–5,000
Large cell carcinoma				
A427	18	National Cancer Institute (ATCC)	3	78–1,250
AHSM	–	Norsk Hydro's Institute, Norway (O. Fodstad)	17	312–5,000
NCI-H460	21, 22	National Cancer Institute (A. F. Gazdar)	17	39–156
Mucoepidermoid carcinoma				
NCI-H292	25	National Cancer Institute (A. F. Gazdar)	17	78–1,250
SCLC[d]				
"Classic"				
NCI-H69	20, 22	National Cancer Institute (A. F. Gazdar)	17/19	156–5,000
NCI-H128	20, 22	National Cancer Institute (A. F. Gazdar)	17/19	1,250–10,000
NCI-H146	20, 22	National Cancer Institute (A. F. Gazdar)	17/19	312–5,000
NCI-H187	22	National Cancer Institute (A. F. Gazdar)	17/19	1,250–10,000
NCI-H249	22	National Cancer Institute (A. F. Gazdar)	17/19	156–5,000
"Variant"				
NCI-H82	22	National Cancer Institute (A. F. Gazdar)	17/19	39–2,500
NCI-H524	22	National Cancer Institute (A. F. Gazdar)	17/19	312–5,000
"Adherent"				
DMS 114	26, 27	Dartmouth Medical School (O. S. Pettengill)	24	39–2,500
DMS 187	26, 27	Dartmouth Medical School (O. S. Pettengill)	24	156–5,000
DMS 273	27	Dartmouth Medical School (O. S. Pettengill)	24	78–1,250
SHP 77	28, 29	University of Pittsburgh (E. R. Fisher)	23	156–5,000
Colon Cancer				
COLO 205	30	Denver Medical Hospital (ATCC)	17	312–5,000
DLD-1	31	Brown University (ATCC)	17	39–625
HCC 2998	–	M. D. Anderson Hospital & Tumor Institute (I. J. Fidler)	16	156–2,500
HCT 116	32	Baylor College (ATCC)	14	39–625
HT-29	19	Memorial Sloan-Kettering Cancer Center (NCI-TB)	17	39–625
LoVo	33	M. D. Anderson Hospital & Tumor Institute (ATCC)	15	39–312
LS 174T	34	Northwestern University Hospital (ATCC)	5	156–5,000
MHC 1544	35	M. D. Anderson Hospital & Tumor Institute (I. J. Fidler)	16	156–10,000
SW 620	36	Scott White Clinic (ATCC)	11	39–625
SW 1116	36	Scott White Clinic (ATCC)	11	156–10,000
WiDr	37	Bureau of Biologics (ATCC)	5	39–1,250
Renal cancer				
A498	18	National Cancer Institute (ATCC)	28	78–2,500
A704	18	National Cancer Institute (ATCC)	28	312–5,000
Caki-1	19	Memorial Sloan-Kettering Cancer Center (MSK)	14	78–5,000
SN12 C	38	M. D. Anderson Hospital & Tumor Institute (I. J. Fidler)	17	39–2,500
SN12 K1	38	M. D. Anderson Hospital & Tumor Institute (I. J. Fidler)	17	39–2,500
UO-31	–	National Cancer Institute (W. M. Linehan)	26	156–1,250
Breast cancer				
HS 578T	39	Naval Biosciences Laboratory (ATCC)	27	312–5,000
MCF7 WT	40	Michigan Cancer Foundation (K. Cowan)	17	39–1,250
MCF7 ADR	41	National Cancer Institute (K. Cowan)	22	39–2,500
MDA-MB-231	42	M. D. Anderson Hospital & Tumor Institute (ATCC)	11	78–5,000
ZR-75-1	43, 44	National Cancer Institute (ATCC)	17	625–5,000
ZR-75-30	43, 44	National Cancer Institute (ATCC)	17	625–5,000

Table 1—*Continued*

Histology cell line	Reference	Institution (Source[a])	Culture medium[b]	MTA inoculation density range[c]
Melanoma				
Hs 294T (A101D)	45	Naval Biosciences Lab (ATCC)	25	625–5,000
LOX	–	Norsk Hydro's Institute, Norway (O. Fodstad)	17	39–625
Malme-3M	19	Memorial Sloan-Kettering Cancer Center (ATCC)	12	312–5,000
RPMI-7951	46	Roswell Park Memorial Institute (ATCC)	3	156–5,000
SK-MEL-1	47	Memorial Sloan-Kettering Cancer Center (ATCC)	3	625–5,000
SK-MEL-2	19	Memorial Sloan-Kettering Cancer Center (ATCC)	3	312–5,000
SK-MEL-5	48	Memorial Sloan-Kettering Cancer Center (ATCC)	3	312–2,500
SK-MEL-28	48	Memorial Sloan-Kettering Cancer Center (ATCC)	3	78–5,000
SK-MEL-31	48	Memorial Sloan-Kettering Cancer Center (ATCC)	4	312–5,000
Ovarian cancer				
A2780	50	National Cancer Institute (T. C. Hamilton)	17	39–2,500
A2780 CP70	50	National Cancer Institute (T. C. Hamilton)	17	78–2,500
CAOV-3	19	Memorial Sloan-Kettering Cancer Center (ATCC)	25	625–5,000
IGROV-1	49	Institute Gustave Roussy, France (J. Benard)	17	78–625
OVCAR 3	50, 51	National Cancer Institute (T. C. Hamilton)	17	156–5,000
OVCAR 4	50, 51	National Cancer Institute (T. C. Hamilton)	17	312–5,000
OVCAR 5	50, 51	National Cancer Institute (T. C. Hamilton)	17	625–5,000
OVCAR 8	–	National Cancer Institute (T. C. Hamilton)	17	78–5,000
SK-OV-3	19	Memorial Sloan-Kettering Cancer Center (ATCC)	13	625–5,000
Prostate cancer				
DU-145	52, 53	Duke University (ATCC)	3	78–2,500
LNCaP	54	Roswell Park Memorial Institute (MSK)	20	39–1,250
PC-3	55, 56	Pasadena Center for Medical Research (M. E. Kaighn)	31	78–5,000
PC-3M	57	National Cancer Institute-FCRF (M. E. Kaighn)	31	78–1,250
UMSCP-1	58	University of Michigan (H. B. Grossman)	9	78–1,250
1013 L	–	University of Minnesota (MSK)	18	1,250–5,000
Leukemia				
CCRF-CEM	59, 60	Children's Cancer Research Foundation (ATCC)	6	1,250–10,000
CCRF-SB	59, 60	Children's Cancer Research Foundation (ATCC)	6	625–10,000
HL-60	61	National Cancer Institute (NCI-TB)	21	78–2,500
K-562	62	University of Tennessee (ATCC)	17	39–156
Molt-4	63	Roswell Park Memorial Institute (ATCC)	17	312–5,000
P388	64	Southern Research Institute (NCI-TB)	17	78–1,250 (4 day)
P388/ADR-Resist	64	Southern Research Institute (NCI-TB)	17	10–312 (4 day)
RPMI 8336	65	Roswell Park Memorial Institute (ATCC)	17	156–5,000
Central nervous system cancer				
SF126	66, 67	University of California (M. L. Rosenblum)	2	78–1,250
SF295	66, 67	University of California (M. L. Rosenblum)	2	39–1,250
SF539	66, 67	University of California (M. L. Rosenblum)	2	156–10,000
SNB19	68, 69	NINCDS (P. L. Kornblith)	29	39–2,500
SNB44	68, 69	NINCDS (P. L. Kornblith)	29	156–5,000
SNB56	68, 69	NINCDS (P. L. Kornblith)	29	39–2,500
SNB75	–	NINCDS (P. L. Kornblith)	29	78–5,000
TE671	70	Children's Hospital, Los Angeles (NCI-TB)	25	156–5,000
U251	71	University of Uppsala, Sweden (NCI-TB)	30	39–5,000
Sarcoma				
A-204	18	National Cancer Institute (ATCC)	14	78–5,000
A673	18	National Cancer Institute (ATCC)	5	156–2,500
HS 913T	46	Naval Biosciences Lab (ATCC)	25	156–5,000
HT1080	72	University of Southern California (ATCC)	5	78–2,500
Te85	73	National Cancer Institute (J. S. Rhim)	10	78–5,000
Head and neck squamous cancer				
UM-SCC-14 B,C	74, 75	University of Michigan (T. E. Carey)	9	156–2,500
UM-SCC-21 A	74, 75	University of Michigan (T. E. Carey)	9	78–5,000
UM-SCC-22 B	74, 75	University of Michigan (T. E. Carey)	9	39–625
Fibroblasts				
CCD-19Lu	46	American Type Culture Collection	30	312–5,000
IMR-90	76	Institute for Medical Research (ATCC)	8	312–5,000
Mar-Bel	46	American Type Culture Collection	30	312–5,000
MCR-5	77	National Institute for Medical Research, UK (ATCC)	1	625–5,000

[a] Cell line sources if other than original investigator were as follows: ATCC, American Type Culture Collection; MSK, Memorial Sloan-Kettering Cancer Center (Walker Laboratory); and NCI-TB, NCI-Division of Cancer Treatment Tumor Bank.

[b] Culture medium formulations recommended by source were as follows: 1 = BME, 10% FBS, Hank's BSS; 2 = EMEM, 10% FBS, NEAA, L-glutamine, gentamicin; 3 = EMEM, 10% FBS, NEAA, pyruvate; 4 = EMEM, 15% FBS, NEAA, pyruvate; 5 = EMEM, 10% FBS, NEAA, Earle's BSS; 6 = EMEM, 10% FBS (modified for suspension); 7 = EMEM, 10% FBS, NEAA, pyruvate, MEM vitamins; 8 = EMEM, 10% FBS, NEAA; 9 = EMEM, 15% FBS, P/S; 10 = EMEM, 10% FBS, P/S; 11 = L 15, 10% FBS; 12 = L 15, 15% FBS; 13 = McCoy's 5A, 15% FBS; 14 = McCoy's 5A, 10% FBS; 15 = Ham's F12, 20% FBS; 16 = Ham's F12, 15% FBS, EGF, transferrin, insulin; 17 = RPMI 1640, 10% FBS; 18 = RPMI 1640, 15% FBS, P/S; 19 = RPMI 1640, hydrocortisone, insulin, transferrin, EGF, selenium; 20 = RPMI 1640, 10% FBS, 2× L-glutamine; 21 = RPMI 1640, 20% FBS; 22 = RPMI 1640, 10% FBS, doxorubicin HCl (5 μM); 23 = RPMI 1640, 9% FBS; 24 = Waymouth's 752/1, 10% FBS, P/S; 25 = DMEM, 10% FBS, 4.5 g/liter glucose; 26 = DMEM, 10% FBS, 4.5 g/liter glucose, HEPES [4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid], P/S/fungizone/gentamicin; 27 = DMEM, 10% FBS, 4.5 g/liter glucose, insulin; 28 = DMEM, 15% FBS; 29 = DMEM/Ham's F12(50:50), 10% FBS, P/S; 30 = CRCM, 10% FBS; 31 = PFMR-4, 3% FBS.

[c] All cells were observed to meet basic quality assurance criteria, were adapted to RPMI 1640, 10% FBS (formulation 17) and were cultivated under a single set of culture conditions (see "Materials and Methods"). Column entries are inoculation densities (cells/200 μl/well) which exhibit exponential or near-exponential growth and a linear, detectable range of absorbance values (minimum > 0.050 and maximum > 0.500 units) following culture for 7 days unless otherwise noted.

[d] SCLC, small cell lung cancer; BME, basal medium (Eagle's); FBS, fetal bovine serum; BSS, balanced salt solution; EMEM, Eagle's minimum essential medium; NEAA, nonessential amino acids; EGF, epidermal growth factor; DMEM, Dulbecco's minimum essential medium; PFMR-4, Pasadena Foundation for Medical

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