CLONOGENIC ASSAY

SCREENING STUDY GROUP

Protocol # 37881

IN VITRO GROWTH INHIBITION OF TUMOUR CELL LINES AND HUMAN BONE MARROW CELLS

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Activated: June 1988

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OBJECTIVES

1- To establish a network of laboratories that will provide rapid results for drugs in at least 8 different cell lines representing a minimum of 5 different histologies.

2- To characterize the different cell lines provided by members of the group.

3- To evaluate the efficacy of new cytotoxic agents in comparison to standard drugs.

4- To determine if new agents have differential activities on specific types of tumors.

ELIGIBILITY FOR THE STUDY

Pilot phase

To participate in the study, a center should be able to perform assays with at least 3 human cell lines of the same tissue origin.
In addition, assays will be performed with the WiDr cell line in the context of the normalization study.

The goal for this phase of the study will be to obtain results with at least 8 cell lines representing breast, lung small cell, lung non-small cell, ovarian and colon cancers.

METHODOLOGY

I. Cell lines
II. Assay system
III. Drug testing
IV. Data analysis

I- CHOICE OF CELL LINES

A) Assays should be performed using the same cell lines from experiment to experiment. Each laboratory should submit an information sheet for each cell line they use.

B) The passage number for the cell lines must be indicated for each assay. Cell lines that are unstable with time in culture should be avoided.

C) Cells used for testing have to be in the log growth phase.

D) The linearity of each assay system with cell number must be established and internal cell number controls provided.

E) Each cell line should be tested regularly for freedom from mycoplasma contamination.

II- ASSAY SYSTEM

EACH LABORATORY WILL USE ITS OWN ASSAY SYSTEM. Please provide the basic data about your system when you first participate. This should be updated whenever any modification to the system is made.

III-DRUG TESTING

For screening purposes continuous exposure will be used exclusively.

A) Adriamycin (doxorubicin) and VP-16 (etoposide) will be used as reference agents to determine the reliability of the assays. In this way, it will be possible to see if there is a shift in the sensitivity of any cell line . Adriamycin can be dissolved in saline at 100 ug/ml and may be kept at -20°C for up to 60 days. VP-16 is provided as a solution by the manufacturer. It should be aliquoted into dark glass ampoules and kept at +4°C (observe manufacturer's expiration date).

Determine the concentrations of drug that inhibit proliferation of each cell line by approximately 90%, 70%, 50%, 30%, and 10% (IC90, IC70, IC50, IC30, IC10). Use these 5 concentrations for subsequent studies.

B) The protocol for other drugs will be adapted each time this study is run.
Bone marrow toxicity (BM) (IC90, IC50, IC10) for each new drug to be tested will be established by at least two of our centers and results will be provided to each participant. Each center participating in the group wide drug testing will have established the IC50 of the reference agents (adriamycin, VP-16) for the cell lines used for consecutive testing of new drugs. In order to test the new drug in concentrations relevant for clinical use, the test concentrations will be determined in relation to the drug concentrations achievable (tolerable) for human bone marrow in vitro. This so-called "normalization" will therefore be based on the corresponding BM IC90, BM IC50 and BM IC10 concentrations . The ratio between the IC50 on the cell line for the standard drug (adriamycin) and its BM IC90 (as maximally tolerated concentration) will be used as the factor for normalizing the new drug concentrations. The same will be done for the other standard drug (VP-16). Only the lower of the ratios (IC50 adriamycin/BM IC90 or IC50 VP-16/BM IC90) will be used.

Example:

BM IC90, IC50, IC10 of drug D	= 3.0; 1.0; 0.2 ug/ml
BM IC90 for adria;VP-16	= 0.03 ug/ml; 0.25 ug/ml
cell line IC50 for adria;VP-16	= 0.18 ug/ml; 2.0 ug/ml
IC50/BM IC90 ratios	= 6 ; 8
thus, the drug D would be tested at:	3.0 ug/ml x 6
	1.0 ug/ml x 6
	0.2 ug/ml x 6

C) All tests will be repeated with cells from 2 different passages. Repeated experiments should be performed at least 2 days apart.
Only one of the reference agents (adriamycin or VP-16) needs to be run in both assays.
Each assay should include control groups for each cell line (without drug) using cell concentrations of 1/3 and 1/10 of the concentration used for the drug tests.

D) If a cell line is too resistant or sensitive at the concentrations tested and an IC50 cannot be determined, the participating center may choose higher or lower concentrations to establish the IC90, IC50, IC10 for the drug. This would eventually be requested by the Data Center, in any case, if the activity of the drug appears to be interesting.