NCI-60 Screening Methodology

NCI-60 Cell One-Dose Screen

General Description

As of early 2007, all compounds submitted to the NCI-60 Cell screen are tested initially at a single high dose (10-5 M) in the full NCI-60 cell panel. Only compounds which satisfy pre-determined threshold inhibition criteria in a minimum number of cell lines progress to the full 5-dose assay. The threshold inhibition criteria for progression to the 5-dose screen were selected to efficiently capture compounds with anti-proliferative activity based on careful analysis of historical DTP screening data. The threshold criteria may be updated as additional data becomes available.

Interpretation of One-Dose Data

The One-dose data is reported as a mean graph of the percent growth of treated cells and is similar in appearance to mean graphs from the 5-dose assay. The number reported for the One-dose assay is growth relative to the no-drug control, and is relative to the number of cells at time zero. This allows detection of both growth inhibition (values between 0 and 100) and lethality (values less than 0). This is the same as for the 5-dose assay, described below. For example, a value of 100 means no growth inhibition. A value of 40 means 60% growth inhibition. A value of 0 means no net growth over the course of the experiment. A value of -40 means 40% lethality. A value of -100 means all cells are dead. Information from the One-dose mean graph is available for COMPARE analysis.

NCI-60 Cell Five-Dose Screen

Compounds that exhibit significant growth inhibition in the One-Dose Screen are evaluated against the 60 cell panel at five concentration levels.
The human tumor cell lines of the cancer screening panel are grown in RPMI 1640 medium containing 5% fetal bovine serum and 2 mM L-glutamine. For a typical screening experiment, cells are inoculated into 96 well microtiter plates in 100 µL at plating densities ranging from 5,000 to 40,000 cells/well depending on the doubling time of individual cell lines. After cell inoculation, the microtiter plates are incubated at 37°C, 5% CO2, 95% air and 100% relative humidity for 24 hours prior to addition of experimental drugs.
After 24 hours, two plates of each cell line are fixed in situ with TCA, to represent a measurement of the cell population for each cell line at the time of drug addition (Tz). Experimental drugs are solubilized in dimethyl sulfoxide at 400-fold the desired final maximum test concentration and stored frozen prior to use. At the time of drug addition, an aliquot of frozen concentrate is thawed and diluted to twice the desired final maximum test concentration with complete medium containing 50 µg/ml gentamicin. Additional 4-fold, 10-fold, or ½ log serial dilutions are made to provide a total of five drug concentrations plus control. Aliquots of 100 µl of these different drug dilutions are added to the appropriate microtiter wells already containing 100 µl of medium, resulting in the required final drug concentrations.
Following drug addition, the plates are incubated for an additional 48 hours at 37°C, 5% CO2, 95% air, and 100% relative humidity. For adherent cells, the assay is terminated by the addition of cold TCA. Cells are

fixed in situ by the gentle addition of 50 µl of cold 50% (w/v) TCA (final concentration, 10% TCA) and incubated for 60 minutes at 4°C. The supernatant is discarded, and the plates are washed five times with tap water and air dried. Sulforhodamine B (SRB) solution (100 µl) at 0.4% (w/v) in 1% acetic acid is added to each well, and plates are incubated for 10 minutes at room temperature. After staining, unbound dye is removed by washing five times with 1% acetic acid and the plates are air dried. Bound stain is subsequently solubilized with 10 mM trizma base, and the absorbance is read on an automated plate reader at a wavelength of 515 nm. For suspension cells, the methodology is the same, except that the assay is terminated by fixing settled cells at the bottom of the wells by gently adding 50 µl of 80% TCA (final concentration, 16% TCA). Using the seven absorbance measurements [time zero, (Tz), control growth, (C), and test growth in the presence of drug at the five concentration levels (Ti)], the percentage growth is calculated at each of the drug concentrations levels. Percentage growth is calculated as:

[(Ti-Tz)/(C-Tz)] x 100 for concentrations for which Ti>/=Tz
[(Ti-Tz)/Tz] x 100 for concentrations for which Ti<Tz.
Three dose response parameters are calculated for each experimental agent. Growth inhibition of 50% (GI50) is calculated from [(Ti-Tz)/(C-Tz)] x 100 = 50, which is the drug concentration resulting in a 50% reduction in the net protein increase (as measured by SRB staining) in control cells during the drug incubation. The drug concentration resulting in total growth inhibition (TGI) is calculated from Ti = Tz. The LC50 (concentration of drug resulting in a 50% reduction in the measured protein at the end of the drug treatment as compared to that at the beginning) indicating a net loss of cells following treatment is calculated from [(Ti-Tz)/Tz] x 100 = -50. Values are calculated for each of these three parameters if the level of activity is reached; however, if the effect is not reached or is exceeded, the value for that parameter is expressed as greater or less than the maximum or minimum concentration tested.