

## EARLY STAGE SUBCUTANEOUS TUMOR MODEL

Murine or human tumor fragments (30 mg) are implanted subcutaneously into the axillary region of pathogen-free immunocompetent or immunodeficient mice, respectively, on experimental day 0. Test agent treatment is initiated either on the day which is historically associated with the start of tumor growth, or when the tumor is palpable. Tumor size and body weights are obtained approximately 2 times per week. Tumor weights are calculated from caliper measurements of tumor dimensions in mm using the formula for a prolate ellipsoid:

$$(L \times W^2)/2 \text{ where } L \text{ is the longer of the 2 measurements}$$

Generally, tumor size is monitored until an upper weight limit of 5000 mg is attained.

The following describes parameters of test agent treatment, activity and toxicity.

Treatment: This section defines the test compound and the dose, route and schedule at which the compound was administered. The routes include intravenous [IV], intraperitoneal [IP], subcutaneous [SC] and oral [PO]. The schedule is shown in abbreviated form. For interpretation, the Q represents 'every', an H represents 'hours' and a D represents 'days'. The day following the comma defines the day post-implant on which the first treatment was administered. Any number(s) in parenthesis represents days on which additional courses of treatment were administered. Thus, Q4D X 3, Day 3 (21) is read as 'every 4 days for a total of 3 treatments with the first treatment given on day 3 and a second course of 3 treatments started on day 21'.

No. of Mice: The total number of mice included in each group when the study was initiated.

No. of Notakes/Spon. Reg.: Number of control mice whose tumors either fail to become established and grow progressively (notakes) or, after a period of growth, decrease to 50% or less of their maximum weight (spontaneous regressions). Treated mice with similar tumor characteristics are defined as notakes/spontaneous regressions or tumor free on the final observation day depending on the number of notakes and spontaneous regressions in the control group.

Drug Deaths: Number of mice in the treatment group which were lost as a result of test agent-related toxicity. A treated animal death is presumed to be treatment related if 1) the animal dies within 15 days of last treatment and a) its tumor weight is  $\leq$  lethal tumor burden in the control mice, or b) its net body weight loss at death is  $\geq$ , by a preselected percentage, than the mean net weight change of the controls at death or sacrifice; or if 2) designated by the screener. Drug deaths are excluded from all calculations relating to antitumor activity.

Max % rel Mean net Wt Loss (day): Maximum percent mean net weight loss. For this, the mean net body weight of each group of mice on each observation day is compared to the mean weight on staging day. Any weight loss which occurs is calculated as a percent of the staging day weight. The day in parenthesis indicates the observation day on which the maximum body weight loss occurred. This information provides an index of the toxicity of the test agent as well as the toxicity of the tumor system itself.

Tumor Free on Day (XX): Number of mice in the group which have no tumor at the end of the experiment. The number in parenthesis indicates the final observation day (Evaluation Day).

Opt. % T/C (day): Percent treated/control is calculated by dividing the median treated tumor weight by the median control tumor weight on each observation day and multiplying by 100. This calculation is performed each day the tumors are measured and the optimum value (minimum), obtained after the first course of treatment, is presented. The day on which this optimum T/C occurs is shown in parenthesis. A T/C % of greater than 40 is considered inactive.

MEDIAN days to X mg: Median of the times (days) required for the treated tumor weights to attain a specified size (e.g., 1000 mg). Selection of the latter is dependent on the tumor growth rate and the treatment period. Tumor free survivors and mice lost to drug-related deaths are excluded from the calculations.

Growth Delay: Expressed as the percentage by which the treated group median tumor weight is delayed in achieving the specified tumor size compared to the controls using the formula:

$$((T-C)/C) \times 100 \quad \text{where T and C are the median times to X mg for the treated and control groups, respectively.}$$

A positive number indicates that the treated tumor reached X mg more slowly than did the control tumor. The greater this positive value, the longer the delay in the treated tumor reaching X mg.

Net Log Cell Kill: An estimate of the number of  $\log_{10}$  units of cells killed by the test agent on the dose, route and schedule used. This value is calculated as:

$$\frac{((T-C) - \text{duration of treatment}) \times 0.301}{\text{Doubling time}}$$

where doubling time is the time required for the tumor to

increase from 200 mg to 400 mg. T and C are the median days to reach the specified tumor size as previously defined.

A log cell kill of 0 indicates the cell population at the end of treatment was the same as at the beginning of treatment while a positive value (>0) indicates the cell population at the end of treatment was less by this number of  $\log_{10}$  units than its size at the beginning of treatment. A log cell kill of +6 indicates a 99.9999% reduction in the cell population.

## ADVANCED SUBCUTANEOUS HUMAN TUMOR XENOGRAFT MODEL

Tumor fragments (30 mg) are implanted subcutaneously into the axillary region of immunodeficient mice on experimental day 0. Tumor growth is monitored and test agent treatment is initiated when the tumors reach a weight range of 100-400mg (staging day). Tumor weights are calculated from caliper measurements of tumor dimensions in mm using the formula for a prolate ellipsoid:

$$(L \times W^2)/2 \text{ where } L \text{ is the longer of the 2 measurements}$$

Tumor size and body weights are obtained approximately 2 times per week. Generally, tumor size is monitored until an upper weight limit of 5000 mg is attained.

Test agent efficacy is evaluated by various parameters calculated from the tumor weights recorded during the experimental period. The following descriptions include explanations of these parameters.

Treatment: This section defines the test compound and the dose, route and schedule at which the compound was administered. The routes include intravenous [IV], intraperitoneal [IP], subcutaneous [SC] and oral [PO]. The schedule is shown in abbreviated form. For interpretation, the Q represents 'every', an H represents 'hours' and a D represents 'days'. The day following the comma defines the day post-implant on which the first treatment was administered (staging day). Any number(s) in parenthesis represents days on which additional courses of treatment were administered. Thus, Q4D X 3, Day 13 (28) is read as 'every 4 days for a total of 3 treatments with the first treatment given on day 13' and a second course of 3 treatments started on day 28'.

No. of mice: The total number of mice included in each group when the study was initiated.

Drug Deaths: Number of mice in the treatment group which were lost as a result of test agent-related toxicity. A treated animal death is presumed to be treatment related if 1) the animal dies within 15 days of last treatment and a) its tumor weight is  $\leq$  lethal tumor burden in the control mice, or b) its net body weight loss at death is  $\geq$ , by a preselected percentage, than the mean net weight change of the controls at death or sacrifice; or if 2) designated by the screener. Drug deaths are excluded from all calculations relating to antitumor activity.

Max % rel Mean net Wt Loss (day): Maximum percent mean net weight loss. For this, the mean net body weight of each group of mice on each observation day is compared to the mean weight on staging day. Any weight loss which occurs is calculated as a percent of the staging day weight. The day in parenthesis indicates the observation day on which the maximum body weight loss occurred. This information provides an index of the toxicity of the test agent as well as the toxicity of the tumor system itself.

Regression: A reduction or loss of tumor burden during the experimental period. Regressions are defined as partial (Part No.) if the tumor weight decreases to 50% or less of the staging day tumor weight without dropping below 63 mg. Complete regressions (Comp No.) are those cases in which the tumor burden falls below measurable levels (<63 mg) but regrows before the end of the experiment.

Tumor Free on Day (XX): Number of mice in the group which have no tumor at the end of the experiment. The number in parenthesis indicates the final observation day (Evaluation Day).

Opt. % T/C (day): Optimal percent treated/control which is actually calculated as a  $\Delta T/\Delta C$ . For this, the staging day median tumor weight of the treated group (Tsd) is subtracted from the median tumor weight on each observation day. This provides the change ( $\Delta$ ) in tumor weight for the treated group ( $\Delta T$ ). The change in tumor weights for the control group ( $\Delta C$ ) is also calculated in this same manner.

$$\% T/C = [(\Delta T/\Delta C) \times 100] \text{ if the } \Delta T > 0$$

$$\text{or } = [(\Delta T/Tsd) \times 100] \text{ if } \Delta T < 0.$$

This calculation is performed each day the tumors are measured and the optimum value (minimum), obtained after the first course of treatment, is presented. The day on which this optimum T/C occurs is shown in parenthesis. The T/C values are interpreted as follows:

T/C % > 40	inactive
T/C % >0 and $\leq$ 40	tumor inhibition
T/C % $\geq$ -50 and $\leq$ 0	tumor stasis
T/C % <-50	tumor regression

Median Tumor Wt (Day XX): Group median tumor weight on the specified day (staging day).

MEDIAN days to X DOUB: Median of the times (days) required for the treated tumor weights to increase by the specified number of doublings. Time to one doubling is the time required for the tumor burden to increase 100%, e.g., a 200 mg tumor grows to a 400 mg tumor. Tumor free survivors and mice lost to drug-related deaths are excluded from the calculations.

Growth Delay: Expressed as the percentage by which the treated group median tumor weight is delayed in achieving the specified number of doublings compared to the controls using the formula:

$$((T-C)/C) \times 100 \quad \text{where T and C are the median times to X doublings for the treated and control groups, respectively.}$$

A positive number indicates that the treated tumor reached XX doublings more slowly than did the control tumor. The greater this positive value, the longer the delay in the treated tumor reaching XX doublings.

Net Log Cell Kill: An estimate of the number of  $\log_{10}$  units of cells killed by the test agent on the dose, route and schedule used. This value is calculated as:

$$\frac{((T-C) - \text{duration of treatment}) \times 0.301}{\text{Doubling time}}$$

where doubling time is the time required for the tumor to increase from 200 mg to 400 mg. T and C are the median days to reach the specified number of doublings as previously defined.

A log cell kill of 0 indicates the cell population at the end of treatment was the same as at the beginning of treatment while a positive value (>0) indicates the cell population at the end of treatment was less by this number of  $\log_{10}$  units than its size at the beginning of treatment. A log cell kill of +6 indicates a 99.9999% reduction in the cell population.