

MODEL: (3M531) Intraperitoneally-Implanted M5076 Sarcoma

Origin of Tumor Line: Arose spontaneously in the ovary of a C57BL mouse at the Papanicolaou Research Institute, Miami, Florida in the laboratory of Dr. W. F. Dunning.

Summary of Test Procedures: A 1:10 tumor brei is implanted i.p. in mice. I.p. test agent treatment begins one day after tumor implant and continues every fourth day for a total of four injections. The parameter is median survival time. Results are expressed as a percentage of control survival time.

ANIMALS: (refer to Protocol 8)

Propagation: C57BL/6 female mice.

Testing: B<sub>6</sub>C<sub>3</sub>F<sub>1</sub> mice (male or female).

Weight: Mice should be within a 3 gm weight range with a minimum weight of 18 gm for males and 17 gm for females.

Sex: One sex is used for all test, titration, and control animals in one experiment.

Source: One source for all animals in one experiment. Exceptions must be noted as comments.

EXPERIMENT SIZE: (refer to Protocol 9)

General Testing: Ten animals per test group.

Control Groups: A minimum of 40 control animals must be used; otherwise, the number of control animals varies according to the number of test groups.

TUMOR TRANSFER: (refer to Protocols 2, 5, and 6)

Propagation:

Tissue:

Fragment: Prepare 2x2x2 mm fragments from 1 to 2 gms s.c. donor tumors.

Time: Day 21.

Site: Implant 1 or 2 fragments s.c. into axillary region with puncture in inguinal region, using a 13 gauge trocar.

Testing:

Tissue:

Brei: Use at least 4 tumors weighing between 1 and 2 gms each. Remove necrotic material, weigh and mince. Estimate gms of tumor needed based upon 33% recovery. Add 4 ml of physiological saline to each gm of tumor. Pour (press) gently through a 40 mesh screen. Break up clumps with a syringe. Pour through second 40 mesh screen. Centrifuge in 50 ml tubes for 5 minutes (600 - 800 x g). (See attached nomograph for computing relative centrifugal force.) Pour off supernatant. To calculate total ml of cells in each graduated tube, add reading at bottom and top of slope of pellet and divide by 2. Add 9 parts of diluent (9 x number of ml of cells). Implant i.p. with 0.5 ml. Use 3 cc syringe with 23 gauge, half inch needle. Agitate suspension in syringe between injections.

Time: Day 21.

Site: Implant i.p. using 0.5 ml of a 1:10 brei.

TESTING SCHEDULE: (refer to Protocols 3 and 4)

Day 0: Implant tumor. Run bacterial cultures (refer to Protocol 7). Prepare materials. Test positive control compound in every experiment. Record deaths daily.

Day 1: Check cultures. Discard experiment if contaminated. Randomize and weigh animals. Treat as instructed. Administer test agent based on initial average group weight.

Day 2: Recheck cultures. Discard experiment if contaminated.

Day 5, 9, 13: Administer test agent.

Day 14: Toxicity day for test animals and second animal weigh day. Control early-death day.

Day 40: Control no-take day.

Day 75: End and evaluate experiment.

QUALITY CONTROL: (refer to Protocol 7)

Schedule the positive control compound (NSC 26271\* at doses of 160 and 80 mg/kg/inj) in every experiment, the regimen for which is Q4D x 4 beginning on Day 1. Solutions of the compound may be prepared in separate aliquots and frozen for later treatments. DO NOT REFREEZE.

The lower T/C limit for the positive control is 150%. The acceptable untreated control median is between 20 and 34 days.

EVALUATION: (refer to Protocols 4 and 11)

The parameter measured is median survival time. Compute average animal body weights for Day 1 and Day 14, compute T/C for all test groups with > 65% survivors on Day 14. A T/C value of < 86% indicates toxicity. A negative body weight change difference (T-C)  $\geq$  4 gms may also be used to evaluate toxicity.

CRITERIA FOR ACTIVITY:

A T/C value of  $\geq$  135% is considered necessary to demonstrate moderate activity. A reproducible T/C value  $\geq$  165% is considered significant activity.

REPORTING OF DATA:

On the final day of testing, prepare final control and test reports.

Assign a Test Status code (TSC) of 33 to any test group the screener considers to be invalid for any reason.

A comment must be provided stating the reason for a TSC of 33, when a non-standard dose is administered, (whether due to a solubility problem or special request), and for poor suspensions.

\*Positive control compound NSC 26271 is Cytosan. CAS RN is 50-18-0.

# NOMOGRAPH FOR COMPUTING RELATIVE CENTRIFUGAL FORCE

