MODEL: (3LO31) Intraperitoneally-Implanted LOX Human Amelanotic Melanoma

Origin of Tumor Line: This line was obtained from Dr. Oystein Fodstad, Department of Biochemistry, Norwegian Radium Hospital, Oslo, Norway.

Summary of Test Procedures: $1 \times 10^6$ cells in ascitic fluid are implanted i.p. in athymic Ncr-nu mice. I.p. test agent treatment starts one day after tumor implant and is repeated every fourth day for a total of three injections. The parameter is median survival time. Results are expressed as a percentage of control survival time.

ANIMALS: (refer to Protocol 8)

Propagation: Athymic Ncr-nu

Testing: Athymic Ncr-nu

Weight: Minimum weight of 18 gm for males and 17 gm for females.

Sex: One sex is used for all tests, titrations, 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: Six animals per test group.

Control Groups: The number of control animals varies according to the number of test groups.

Titrations: Each control is to include titrations of $1 \times 10^7$ to $1 \times 10^3$ cells, inclusive, with ten animals per level.

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

Propagation:

Tissue:

Suspension: Prepare diluted ascitic fluid containing $1 \times 10^7$ cells (approximately 0.1 ml). (Refer to the following section entitled Preparation of Cell Suspension).

Time: Days 7-11 (Harvest should be done between the time the animal becomes obviously symptomatic and before the ascitic fluid becomes dark red.)

Site: Implant $1 \times 10^7$ cells/animal injecting approximately 0.1 ml per mouse I.P. using a 0.5 inch 23 gauge needle with a 1 ml tuberculine syringe. Site of injection should be chosen to avoid vital organs.

Testing:

Tissue:

Suspension: Prepare 0.1 ml of diluted ascitic fluid containing $1 \times 10^6$ cells. (Refer to the following section entitled Preparation of Cell Suspension).

Time: Days 7-11 (Harvest should be done between the time the animal becomes obviously symptomatic and before the ascitic fluid becomes dark red.)

Site: Implant 1 x $10^6$ cells/animal injecting 0.1 ml per mouse I.P. using a 0.5 inch, 23 gauge needle with a 1 ml tuberculin syringe. Site of injection should be chosen to avoid vital organs.

PREPARATION OF CELL SUSPENSION:

Use donor tumor when the abdomen becomes distended (usually Days 7 through 11). Using a sterile 5 ml syringe, withdraw ascitic fluid asceptically through the abdominal wall from which the skin has been removed. Use a minimum of 3 mice. Collect at least 3 ml of ascitic fluid. Pool fluid in a sterile glass container held in an ice bath.

Cell Count: Use physiological saline for dilutions. Use serological pipettes with rubber bulb attachment. Cell suspensions should be swirled and mixed by aspirating the solution into and out of the pipette several times before withdrawing an aliquot.

Dilutions:

Suspension A: 0.5 ml of pooled ascitic fluid plus 4.5 ml of physiological saline.

Suspension B: 0.5 ml of Suspension A plus 4.5 ml of physiological saline.

Suspension C: 1.0 ml of Suspension B plus 4.0 ml of physiological saline. Suspension C is a 1:500 dilution and should be used to make the cell count as follows:

1. Agitate Suspension C and withdraw 0.5 ml with a 1-cc syringe and a 23 gauge needle.
2. Agitate and allow several drops to flow out, then fill both chambers of the hemacytometer.
3. Count only intact nucleated cells using 100x to 400x magnification.
4. Assuming the use of an AO STD or comparable hemacytometer, count 4 large squares in both chambers, being sure to establish and follow a convention for inclusion of cells that fall on lines.
5. The cell count and the dilution ratio may be read directly from the accompanying table. To use the table, divide the total number of cells in both chambers (refer to Step 4) by 2.

These values may also be calculated directly from the numbers of cells in both hemacytometer chambers as follows:

$$\frac{\text{Total No. of Cells in Both Hemacytometer Chambers}}{2} \times 2.5 \times 500 \times 1000^{**} = \text{Cells/ml of the Undiluted Ascitic Fluid}$$

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**2.5: Correction factor to convert count to cells per cubic mm.
500: Dilution factor.
1000: Converts $mm^3$ to ml.

Note: Once the cell count has been determined, there is no further need for Suspensions A, B, or C. The cell suspension for inoculation will be made from the original undiluted ascites from which Suspension A was made.

The dilution factors required to prepare a suspension of cells that contain the highest cell number required in 0.1 ml may be calculated as shown in the following example:

$$\frac{\text{10,000,000 (Cells/ml Required for 1 x } 10^6 \text{ in 0.1 ml Implant)**}}{\text{85,000,000 (Cell Count/ml of Ascitic Fluid)}} = \frac{\text{X ml (ascites)}}{\text{10 ml (total volume)}}$$

$$85X = 100$$
$$X = 1.18 \text{ ml}$$

Add 1.18 ml of ascitic fluid to 8.82 ml of physiological saline for a suspension of 1 x $10^6$ cells in 0.1 ml.

Inoculation: Sterile procedures should be followed to dilute the ascitic fluid to obtain Suspension 1 and subsequent dilutions.

Suspension 1: Highest level required; 1 x $10^6$ cells in 0.1 ml.

Suspension 2: Add 1 part of Suspenion 1 to 9 parts of physiological saline = 1 x $10^5$ cells in 0.1 ml.

Suspension 3: Add 1 part of Suspension 2 to 9 parts of physiological saline = 1 x $10^4$ cells in 0.1 ml.

Suspension 4: Add 1 part of Suspension 3 to 9 parts of physiological saline = 1 x $10^3$ cells in 0.1 ml.

Use sufficient numbers of sterile syringes and needles so that that no syringe will be refilled from the pool of donor fluid. No more than 60 minutes should elapse from the time fluid is taken from the donor until it is implanted in all of the recipient animals. For titrations, inoculate the lowest level first, then proceed to inoculate each higher level.

Note: Although the LOX amelanotic melanoma is passaged from ascites-free cells, there will be some solid tumor formation in the peritoneal cavity. This solid tumor formation becomes more pronounced with time after implant. Also, this tumor appears to produce a mucus-like condition that results in thick ascitic fluid. It may be necessary to inject 1 or 2 ml of physiological saline in order to facilitate harvest.

**For stock passage and titrations, this value should be 100,000,000 (cells/ml required for $10^7$ in approximately 0.1 ml implant).

TESTING SCHEDULE: (refer to Protocols 3 and 4)

Day 0: Implant tumor. Run bacterial cultures (refer to Protocol 7 and Instruction 348). Prepare materials. Test positive control compound in every experiment. Refer to Protocol 10 or Instruction 361 for instructions on randomization. Record deaths daily.

Day 1: Check Cultures. Discard experiment if contaminated. Weigh animals individually and record weights. Treat as instructed. Administer test agent based on individual body weight (0.1 ml/10 gm).

Day 2: Recheck cultures. Discard experiment if contaminated.

Day 5: Administer test agent based on individual body weight.

Day 9: Administer test agent based on individual body weight.

Day 10: Control early-death day, test toxicity day. Weigh Day 2. Record individual body weights.

Day 40: Control no-take day.

Day 60: Final Evaluation Day.

QUALITY CONTROL: (refer to Protocol 7)

Schedule the positive control compound (NSC 19893* at doses of 75 and 50 mg/kg/injection) in every experiment, the regimen for which is Q4D x 3 beginning on Day 1. The lower T/C limit for the positive control is 140%. The acceptable untreated control median survival time is 15 - 21 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 10, compute T/C for all test groups with > 65% survivors on Day 10. A T/C value < 86% indicates toxicity. An excessive body weight change difference (test minus control) may also be used in evaluating toxicity.

CRITERIA FOR ACTIVITY:

An initial T/C $\geq$ 140% is considered necessary to demonstrate moderate activity. A reproducible T/C value $\geq$ 200% 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 nonstandard dose is administered (whether due to a solubility problem or special request) and for poor suspensions.

*Positive control compound NSC 19893 is 5-FU. CAS RN is 51-21-8.