

PROCEDURES FOR LIFESPAN ASSAY USING HUMAN TUMOR XENOGRAFTS IMPLANTED INTRAPERITONEALLY

ANIMALS:

Testing: Athymic Swiss (Cr:NIH9SO-nu) or random bred (NCr-nu) mice.
Weight: Mice should have a minimum weight of 18 gm for males and 17 gm for females.
Age: Record age of mice.
Sex: One sex is used for all test and control animals in one experiment.
Source: One source, if feasible, for all animals in one experiment.
Exceptions to be noted as comments.

EXPERIMENT SIZE:

General Testing: Ten animals per test group and 20 animals per control group. (The groups may be reduced to 6 per test group and 12 per control group when it is necessary to conserve animals).

TUMOR TRANSFER:

BY TUMOR BREI: (1) Sacrifice a minimum of 3 donor mice and immerse in disinfectant. Attach to cork board. Remove abdominal skin.
(2) Inject the peritonea cavity with one or two ml of a physiological saline or a nutrient mixture.
(3) With a second syringe (5 cc with a 18-gauge needle) withdraw as much ascitic fluid as possible.
(4) Place the collected ascites in graduated centrifuge tubes (15 ml) and centrifuge in an International Clinical Centrifuge at 2000 RPM for 5 min. Pour off supernatant. Determine volume of packed cells and resuspend in three times that amount of saline or nutrient mixture.

Testing: Implant one ml of a 25 % w/v cell suspension, IP. Include 2 to 4 additional groups with 1:10 serial dilutions of the tumor preparation for determination of doubling time.

BY COUNTED CELL SUSPENSION: (1) Sacrifice a minimum of 3 donor mice, immerse in a disinfectant and attach to a cork board. Remove abdominal skin.
(2) Using a 5 ml syringe, withdraw ascites (3 ml or more) through the abdominal wall.
(3) Pool fluid in sterile glass container in ice bath.

(4) Perform cell count.

(5) Using saline as diluent, prepare 1:10 dilutions for titration with cell concentrations from 1×10^3 to 1×10^7 cells.

Testing:

Implant one ml of counted cell suspension, ip.

TESTING SCHEDULE:

Day 0: Implant tumor. Run bacterial cultures. Randomize mice. Record deaths daily. Prepare test material.

Day 1: Check cultures. Discard experiment if contaminated. Weigh mice, record. Treat as outlined in "In Vivo Characterization and Reference Data for IP Human Tumor Xenografts".

Day 2: Check cultures. Discard experiment if contaminated.

EVALUATION:

The parameter measured is median survival time.

***In Vivo* Characterization and Reference Data for IP Human Tumor Xenografts**

<u>Study Code</u>	<u>Tumor Line</u>	<u>Implant</u>	<u>Rx Schedule</u>	<u>Evaluation Day</u>	<u>DT Days</u>	<u>Lifespan</u>	<u>Schedule</u>	
							<u>Version</u>	<u>Date</u>
3HL31	HL-60(TB)	10 ⁷	Days 1,5,9	50-70*	2.6-4.9	20-25	A	2/28/91
3LO31	LOX IMVI	10 ⁶ (0.1 ml)	Days 1,5,9	60	1.2-2.5	15-21	374D	7/10/86
3OC31	OVCAR-3	25% w/v (1ml)	Days 7,14,21	150*	1.8-9.2	ca 50	394	4/03/87

*Evaluation day is flexible to obtain maximum information on active compounds.
 Mean animal weights should be obtained on a weekly or biweekly basis.