

GENERAL INSTRUCTIONS FOR EARLY TREATMENT OF
SUBCUTANEOUSLY IMPLANTED XENOGRAFTS (72 PROTOCOL)

ANIMALS:

Propagation: Athymic random bred (NCr-nu) mice.

Testing: Athymic random Bred (NCr-nu) mice.

Weight: Mice should have a minimum weight of 18 g for males and 17 g for females.

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 in comments.

EXPERIMENT SIZE:

General Testing: 6-10 animals per test group depending upon tumor system.

Control Group: A minimum of 20 control animals must used.

TUMOR TRANSFER FOR PROPAGATION AND TESTING:

Fragment: Prepare a 30 mg (acceptable range 20-40 mg) fragment from 300-1000 mg sc donor tumor without ulcerations.

Site: Implant 30 mg fragment sc into axillary region with puncture in inguinal region.

TEST REQUIREMENTS:

Randomization: Mice will be randomized by body weight rather than by tumor weight since tumors may not be detectable at the time drug treatment is initiated. This will randomize any effect of higher body weights on the tumor take rate across the experimental groups.

First Treatment: Measure tumors (if detectable) to nearest 1.0 mm. Record tumor measurements (mm) and animals weights (g) for individual mice (Weigh Day 1). Unless specified otherwise, treatment will be Q4D X 3 starting Day 3 post-implantation. This treatment may be adjusted for individual tumor systems and compounds (See Table 1).

Deaths: Record deaths daily.

Treatments: Administer test agent based on the individual body weights on the specified days of treatment by the specified treatment route.

Toxicity: Record individual animal weights on the tumor measurement days. If mice have a 20% or greater loss in body weight not associated with tumor growth, the dose should be considered toxic. Tumor measurements should be performed 2X weekly.

Early Sacrifice: If individual tumors approach 5 g or more, the mice should be sacrificed and tumor dimensions and animal weights recorded.

Evaluation Day: At least 14 days after the last treatment, unless tumor growth requires earlier sacrifice, end and evaluate experiment. Record individual tumor measurements and animal weights.

EVALUATION OF ACTIVITY:

The following parameters will be recorded/calculated:

- number of tumor-free animals
- number of drug-related deaths
- number of no takes
- optimal T/C%
- %T-C/C
- median days to achieve a defined tumor weight
- net log cell kill

TABLE I

Tumor acceptable for Early Subcutaneous Treatment Protocol (72 Protocol)

TUMOR	RATING	TREATMENT SCHEDULE (Treatment start day)	MEASUREMENTS/WEEK
LUNG			
NCI-H460	B	qdx5 (2)	2
NCI-H23	B	q4dx3 (3)	2
DMS-273	B	qdx5 (2)	2
NCI-H322M	G	q4dx3 (4)	2
EKVX	G	q4dx3 (3)	2
NCI-H522	G	qdx5 (3)	2
RENAL			
CAK1-1	B	qdx5 (2)	2
RXF-393	B	qdx5 (2)	2
A498	G	q4dx3 (3)	2
SN121C	G	q4dx3 (4)	2
RXF-631	G	q4dx3 (3)	2
COLON			
HCT-116	B	qdx5 (2)	2
SW-620	B	qdx5 (3)	2
HCT-15	B	qdx5 (5)	2
KM-12	B	q4dx3 (3)	2
HT29	G	q7dx3 (3)	1
HCC-2998	G	q4dx3 (3)	2
KM20L2	G	q4dx3 (3)	2
COLO-205	G	q4dx3 (2)	2
DLD-1	G	q4dx3 (4)	2
MELANOMA			
LOX IMVI	B	qdx5 (2)	2
SK-MEL-28	B	qdx5 (3)	2
UACC-62	G	q4dx3 (4)	2
UACC-257	G	q7dx3 (3)	1
M14	G	q7dx3 (4)	1
OVARIAN			
OVCAR-5	B	q4dx3 (6)	2
SK-OV-3	B	q4dx3 (6)	2
CNS			
SNB-75	B	q4dx3 (6)	2
SF-295	B	qdx5 (2)	2
U251	G	q4dx3 (4)	2
PROSTATE			
PC-3	B	qdx5 (2)	2
LNCAP	B	qdx5 (2)	2

Rating: B - best of available sc lines for early treatment G - good lines for use in the early treatment protocol

PERTINENT DETAILS FOR "72" XENOGRAFT PROTOCOLS ON CHART

<u>TUMOR</u>	<u>DETAILS</u>
CL72	PROPAGATION: Donor tumor weight is from 500-800 mg (approximately 10-14 days after implant). Inoculate in axillary region with incision in inguinal region. Weigh both animals and tumors on staging day and on termination of experiment. Terminate experiment on day 20.
JA72	PROPAGATION: Donor tumor weight is from 200-500 mg (approximately 20-28 days after implant). Inoculate in axillary region with incision in inguinal region. Weigh both animals and tumors on staging day and on termination of experiment. Staging Day is ca Day 20 to 28. Terminate experiment on Staging Day + 16.
JC72	PROPAGATION: Donor tumor weight is from 200-500 mg (approximately 18-25 days after implant). Inoculate in axillary region with incision in inguinal region. Weigh both animals and tumors on staging day and on termination of experiment. Staging Day is ca Day 18 to 25. Terminate experiment on Staging Day + 12.
JP72	PROPAGATION: Donor tumor weight is from 200-500 mg (approximately day 9-13 days after implant). Inoculate in axillary region with incision in inguinal region. Weigh both animals and tumors on staging day and on termination of experiment. Staging Day is ca Day 9 to 13. Terminate experiment on Staging Day + 12.