Drug Studies Using Mouse Models

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Why should the taxpayers fund our work?

Cancer is the second leading cause of death among Americans. It accounts for one of every four deaths in the US. More than 565,000 Americans—or more than 1,500 people a day— die annually from cancer. Close to 1.4 million new cases are diagnosed each year. This estimate does not include pre-invasive cancer or the more than 1 million cases of non-melanoma skin cancer expected to be diagnosed.

Why do we use mouse models?

To prevent, diagnose and treat human disease

- genetics
- progression
- prevention
- diagnostics
- therapeutics
- physiologically complex vs in vitro studies
- cost effective
- develop human clinical protocols

What models are available?

- Spontaneous
- Virus-induced
- Transgenic
- Knock-out/in
- Induced/carcinogens
- Transplanted

Spontaneous Models

These allow the study of the biological history of natural disease. They can be applied to many types of studies.

- Natural history is most "normal"
- Random tumors
- Difficult to predict occurrence
- Must monitor mice through lifespan ~ 2 yr
- Large number of animals may be required for a small experiment
- Tumors may be heterogeneous
- Significant animal holding space needed

Virus-Induced Models

Early studies of cancer, both as a disease state and for therapeutic intervention, used virus-induced models. These models continue to be applied in a variety of studies.

- Classic models of virus-induced leukemia:
  - Rauscher
  - Moloney
  - LP-BM5
  - Friend
- Non-leukemic tumors including:
  - Mammary tumors due to MMTV
  - Thymomas of AKR mice

- High tumor occurrence rate
- Predictable time to tumor development
- Well-characterized disease states/natural history
- May not accurately reflect the natural history of human tumors

Transgenic Models

Transgenic models aid our understanding of the genetics of cancer and are being pursued as models for intervention.

- Many available through commercial and collaborative arrangements
  - http://emice.nci.nih.gov/emice/mouse_models
  - http://mouse.ncifcrf.gov/
  - http://jaxmice.jax.org/query/f?p=205:1:1510299228434659165

- Not all patents have expired - check with OTT regarding legalities
- Remember MTAs are needed to receive or ship material that may have patent or licensing rights. This also applies to mice.

- Rate of tumor occurrence may be low
- Time to tumor occurrence may be difficult to predict
- Breeding schemes may be complex e.g., 3 and 4 way crosses
- Genetic error(s) are generally well characterized
- Disease may follow a more natural course e.g., metastatic lesions, time to significant tumor burden may more accurately mimic the human

Induced/Carcinogens

These models aid studies of the local, systemic, and environmental factors that influence tumor susceptibility, growth, and progression.

- **Epithelial tumorigenesis**
  - http://www.cshprotocols.org/cgi/content/full/2007/18/pdb.prot4837
  - 7,12-dimethylbenz[a]anthracene [initiator mutagen] followed by multiple applications of 12-*O*-tetradecanoylphorbol-13-acetate [pro-inflammatory]
- **GI tumorigenesis**
  - 1,2 dimethlhydrazine-2-HCl
  - azoxymethane
- **Sarcoma induction**
  - Methylcholanthrene
- **Lung tumorigenesis**
  - nitrosamine 4- (methyl-nitrosamino)-1-(3-pyridyl)-1-butanone

Induced/Carcinogens

- Time to tumor development may be variable
- Mimics some human diseases very well
- Requires technical work with known carcinogenic agents thus special handling equipment/facilities/training required
- Provides the entire natural history of tumor development for study

Transplanted Models

These models are commonly applied in studies of genetics, diagnostics, and medical interventions.

- Tissue Source
  - Syngeneic - same inbred strain – e.g., B16 tumors in C57Bl/6 mice
  - Allogeneic - same species, different strain (genetically diverse) – e.g., M5076 sarcomas in athymic mice
  - Xenogeneic - different species e.g., human tumors grown in immunocompromised mice
- Implant Site
  - Orthotopic
  - Heterotopic
- Endpoints

Transplanted Models

- Easy to control time of tumor occurrence
- Many tumor types/lines available
- Well accepted models
- Do not accurately recapitulate human disease
- Metastatic lesions are difficult to find
- Tumor growth rates may preclude multiple treatment cycles

Application of mouse models to preclinical drug development

Discovery & Development

production

In vitro studies

New therapy

efficacy trials

Pharmacology & toxicology

In Vivo Efficacy Models

- Human Tumors
  - Hollow fiber
  - Subcutaneous
  - Intravenous
  - Intraperitoneal
  - Orthotopic
    - Mammary fat pad
    - Intracranial
    - Intrarenal
    - Intrahepatic
- Rodent Tumors
  - Subcutaneous
  - Intravenous
  - Intraperitoneal
  - Orthotopic
  - Metastatic
  - Transgenic
  - Knock-in/out
  - Induced
  - Spontaneous

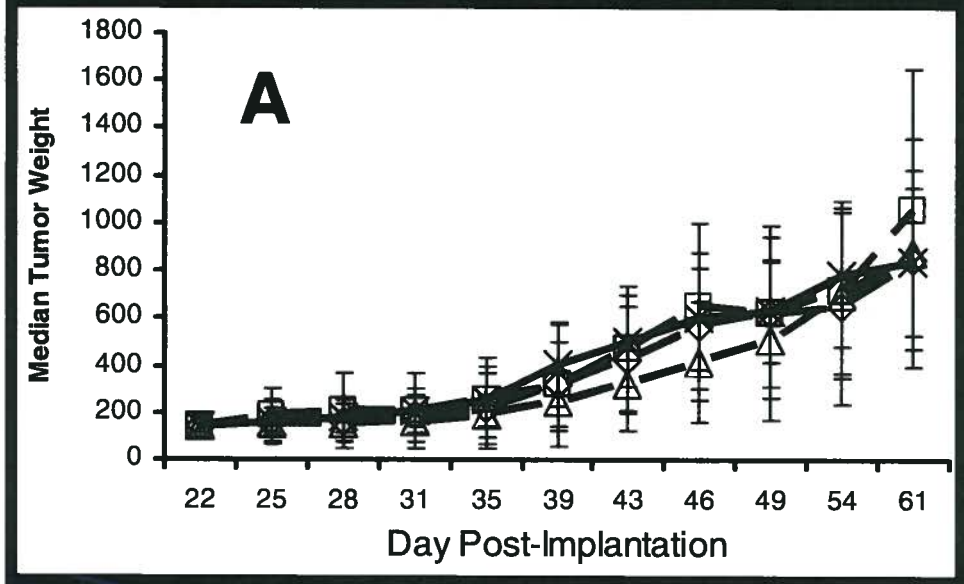
Issues faced during Efficacy Evaluations

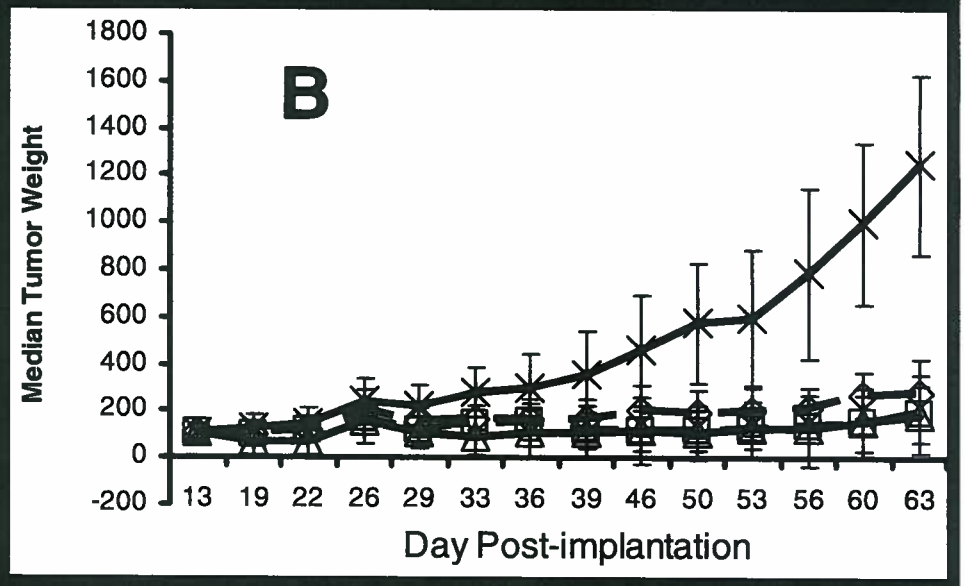
- Model
- Vehicle, formulation, stability
- Dose, route and schedule
- Experimental protocol
- Endpoints

Model Selection

- What are you assessing?
- Which type of model is most appropriate?
- Is the treatment designed to:
  - impact the tumor chemically
  - impact the tumor genetically
  - impact the stroma e.g., vasculature
  - impact the immune system
  - act as an adjuvant
  - synergize with known drugs
  - interact with specific proteins

Model Selection





100% sesame oil

45 mg/kg tamoxifen

22.5 mg/kg tamoxifen

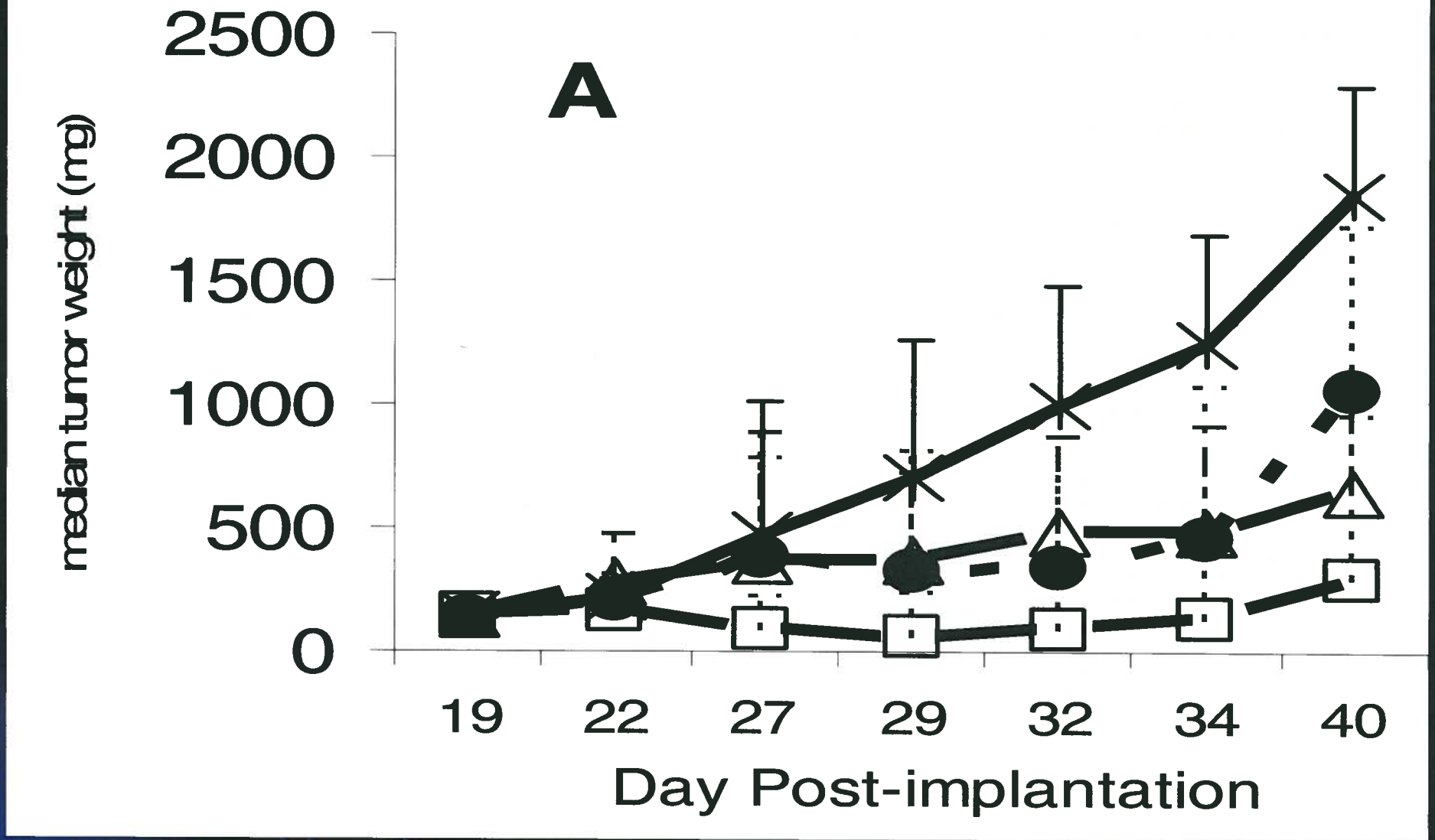
11.25 mg/kg tamoxifen

Experimental Protocol

- When will treatment start?
- When will treatment end?
- Will samples be collected for ex vivo evaluation?
- Will tumors be monitored visually, by imaging techniques?
- What will result in terminating the experiment?

Dose, Route, Schedule

- What is published/prior knowledge?
- What is proposed/expected mechanism?
- How much exposure is required for effect?
- Is the material soluble/stable in aqueous solution?
- What routes of administration are technically feasible?
- Options
  - MTD?
  - IP, IV, SC, PO?
  - QDx?; BIDx?; TIDx?



—×— vehicle

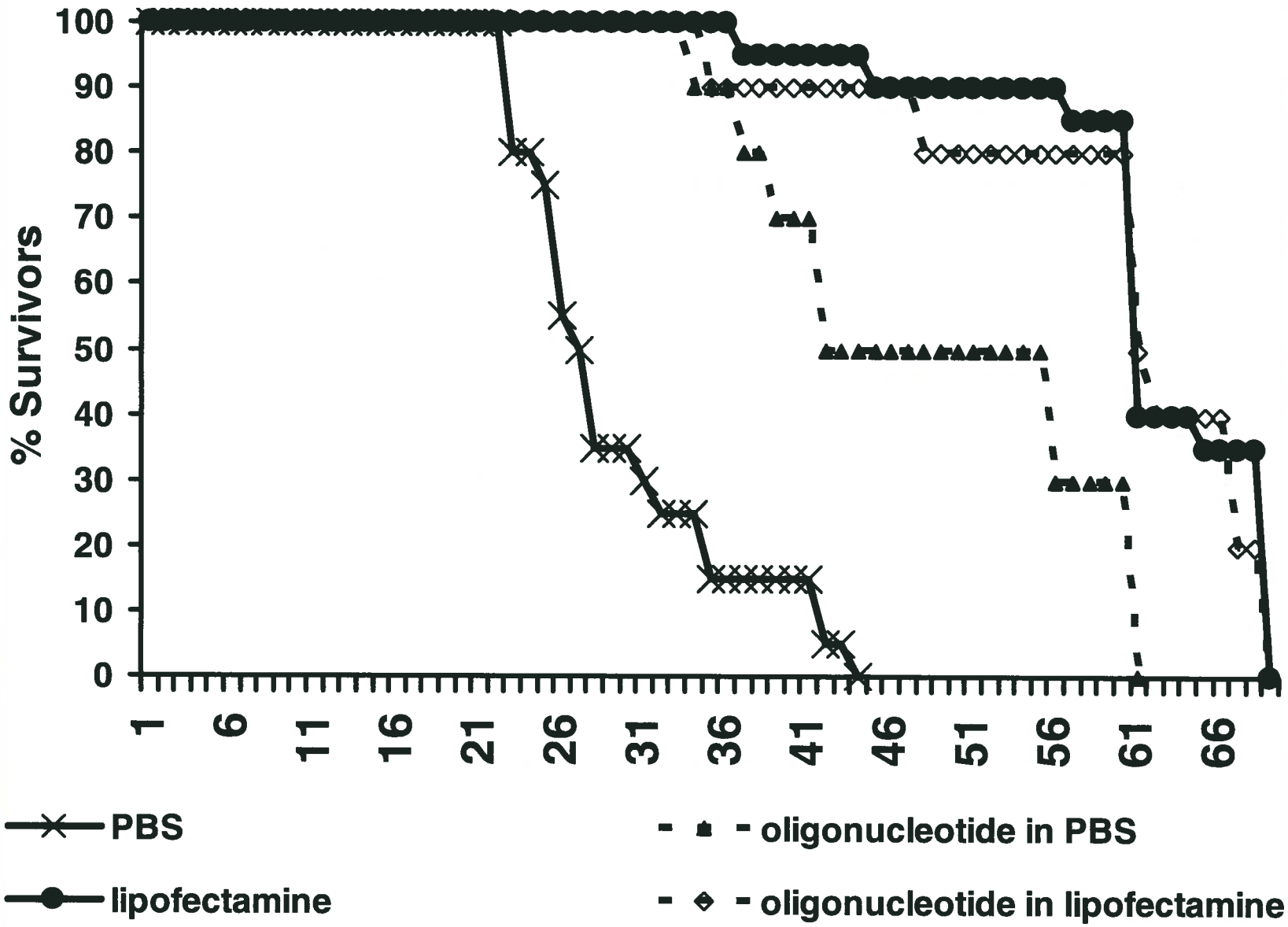
—Δ— temozolomide 200 mg/kg x 3

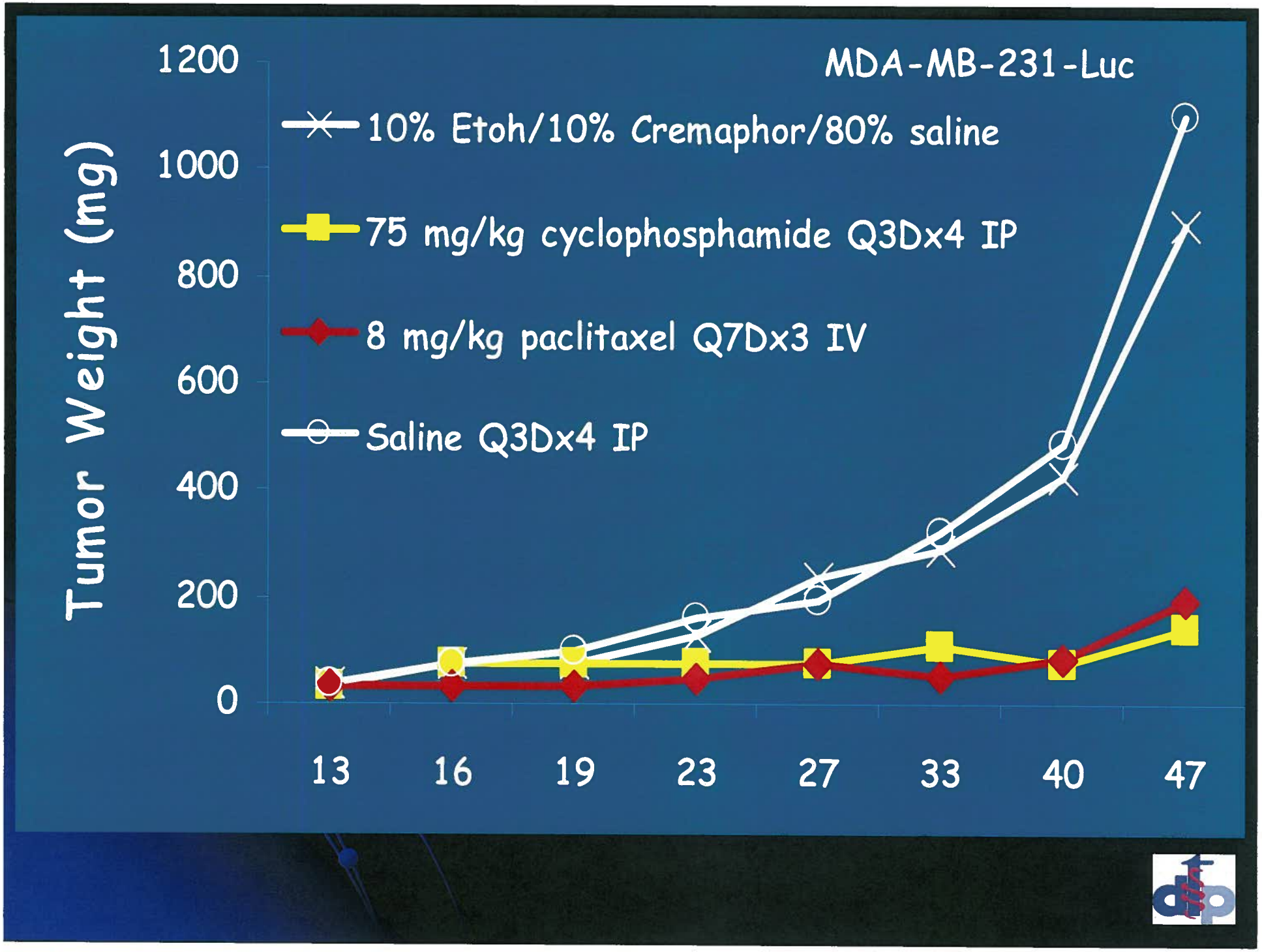
—□— temozolomide 400 mg/kg

—●— topotecan 1 mg/kg QDx10

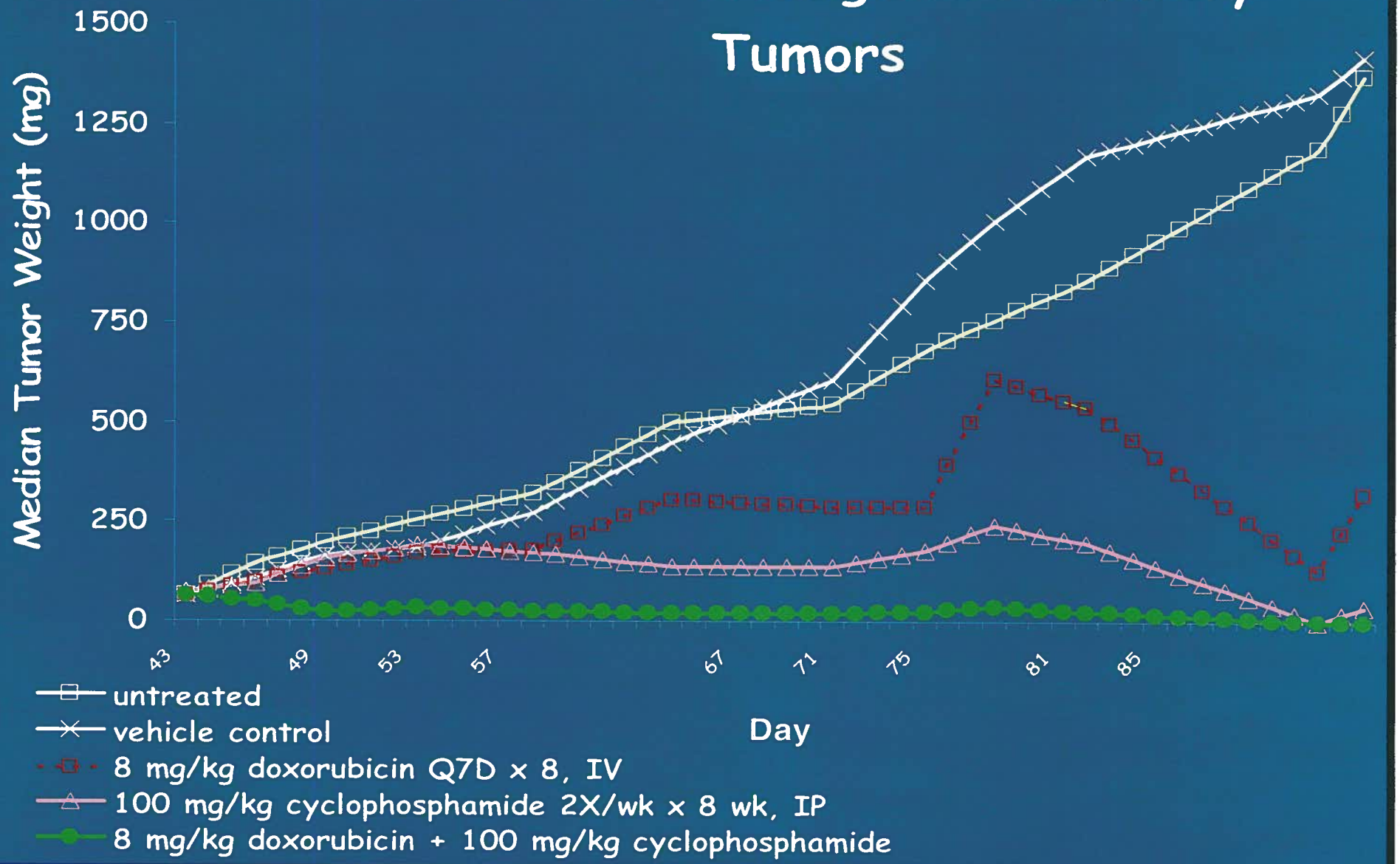
Endpoints

- Tumor size
- Weight loss
- Time to sacrifice
- Imaging
- Pre-defined time of termination
- Time post-treatment

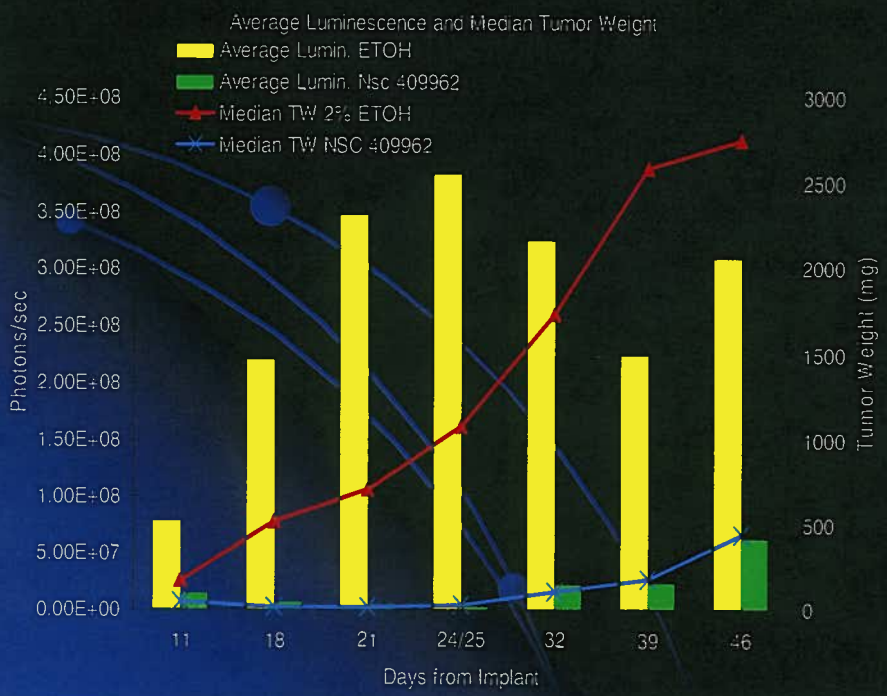

% Survivors
100
90
80
70
60
50
40
30
20
10
0
1
6
11
16
21
26
31
36
41
46
51
56
61
66
PBS
lipofectamine
oligonucleotide in PBS
oligonucleotide in lipofectamine


MDA-MB-231-Luc
Tumor Weight (mg)
1200
1000
800
600
400
200
0
10% Etoh/10% Cremaphor/80% saline
75 mg/kg cyclophosphamide Q3Dx4 IP
8 mg/kg paclitaxel Q7Dx3 IV
Saline Q3Dx4 IP
13
16
19
23
27
33
40
47

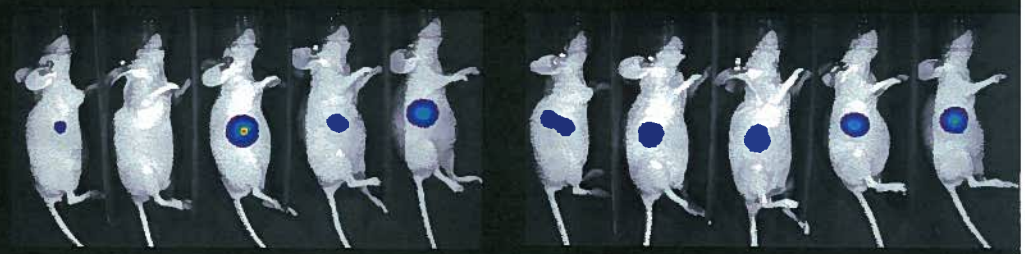
MMTV-PvT Transgenic Mammary Tumors



Measuring antitumor drug activity using bioluminescence vs. tumor weights

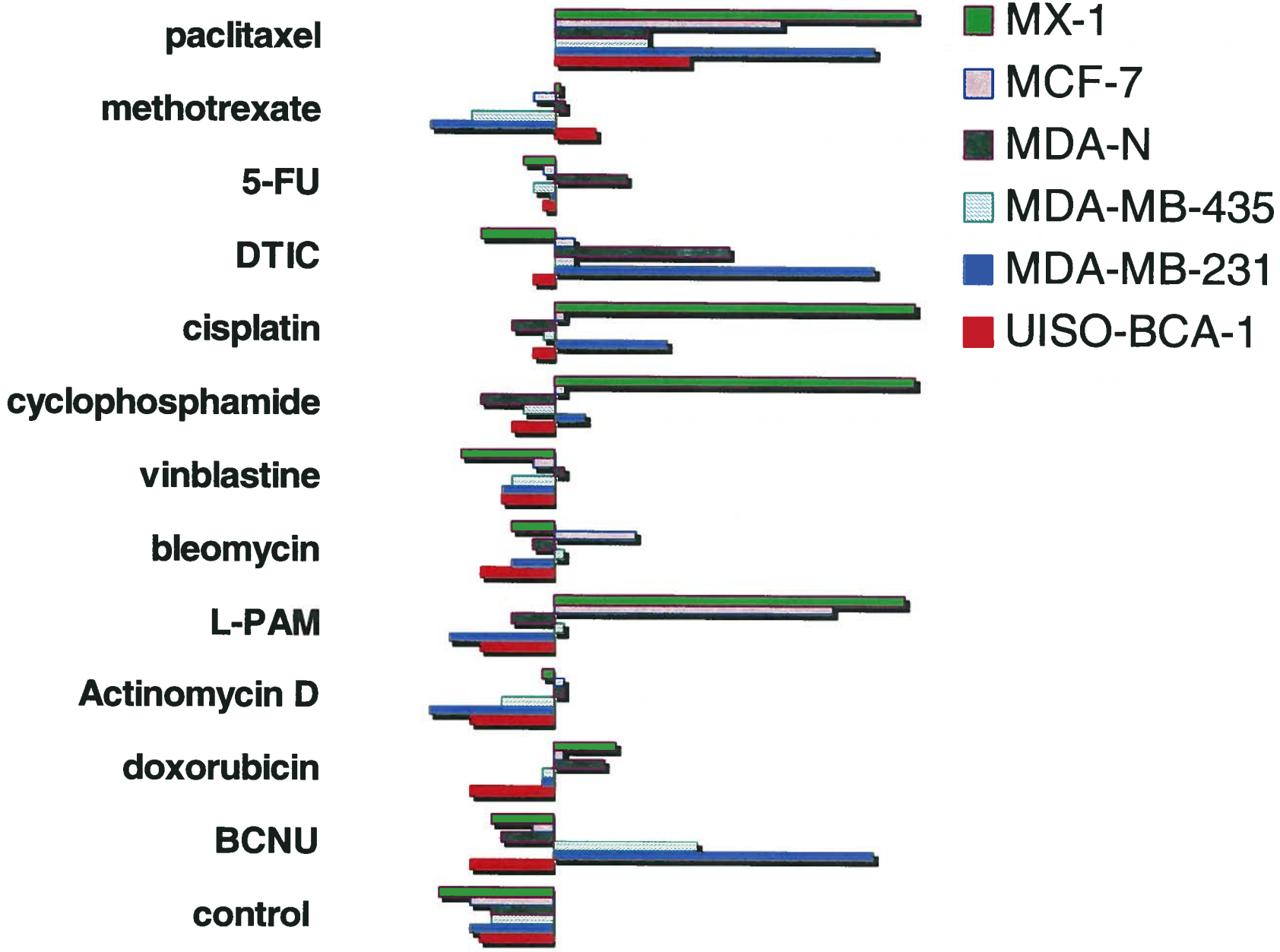


Vehicle BCNU

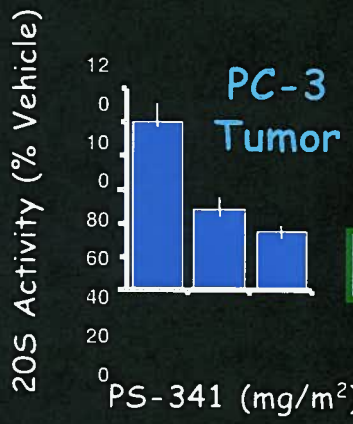


Prior to Treatment (Day 6)

7 days after third treatment (Day 21)


paclitaxel
methotrexate
5-FU
DTIC
cisplatin
cyclophosphamide
vinblastine
bleomycin
L-PAM
Actinomycin D
doxorubicin
BCNU
control
MX-1
MCF-7
MDA-N
MDA-MB-435
MDA-MB-231
UISO-BCA-1

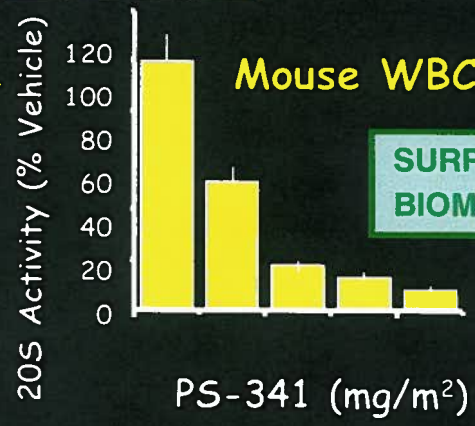
In Vivo Studies



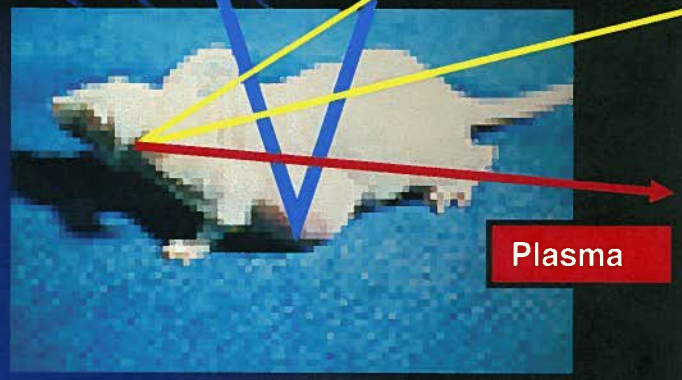
BIOMARKER

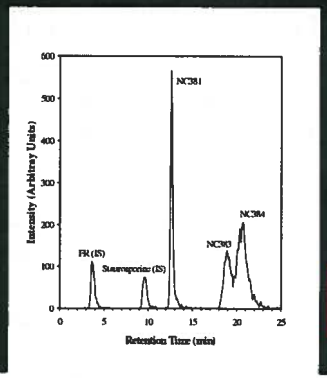
Tumor RNA

GENOMICS



SURROGATE BIOMARKER

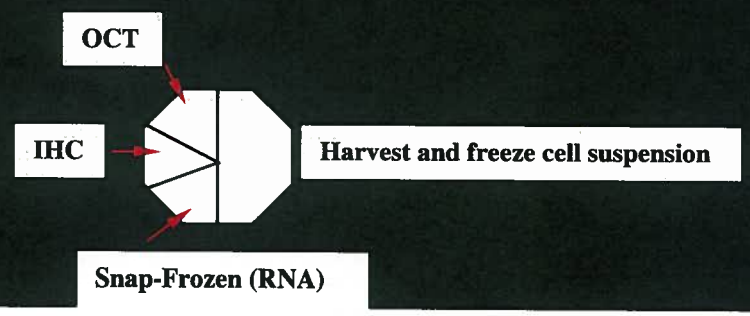


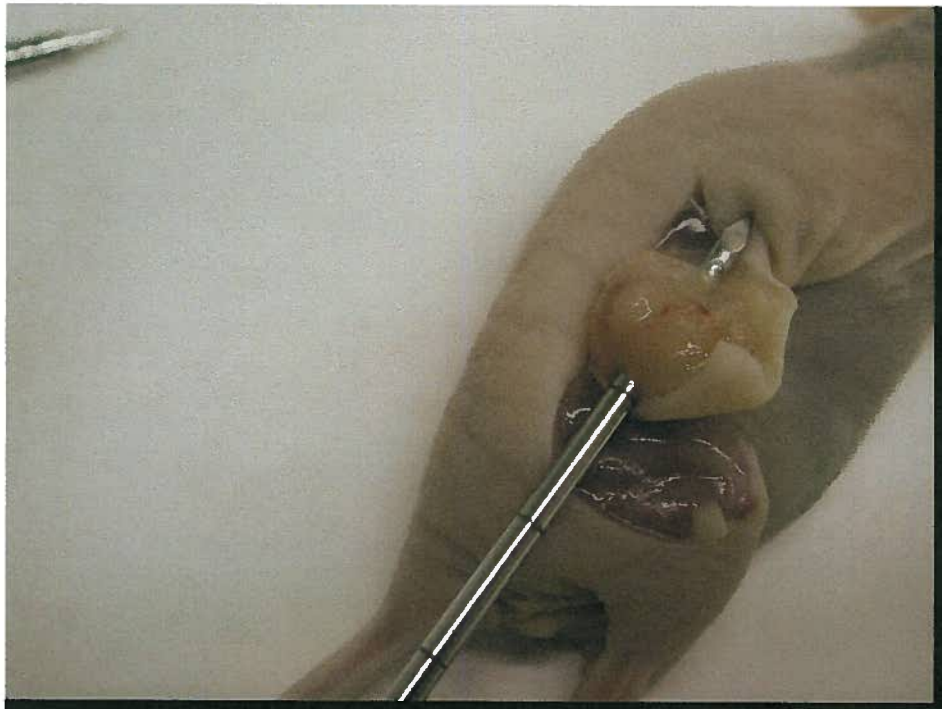


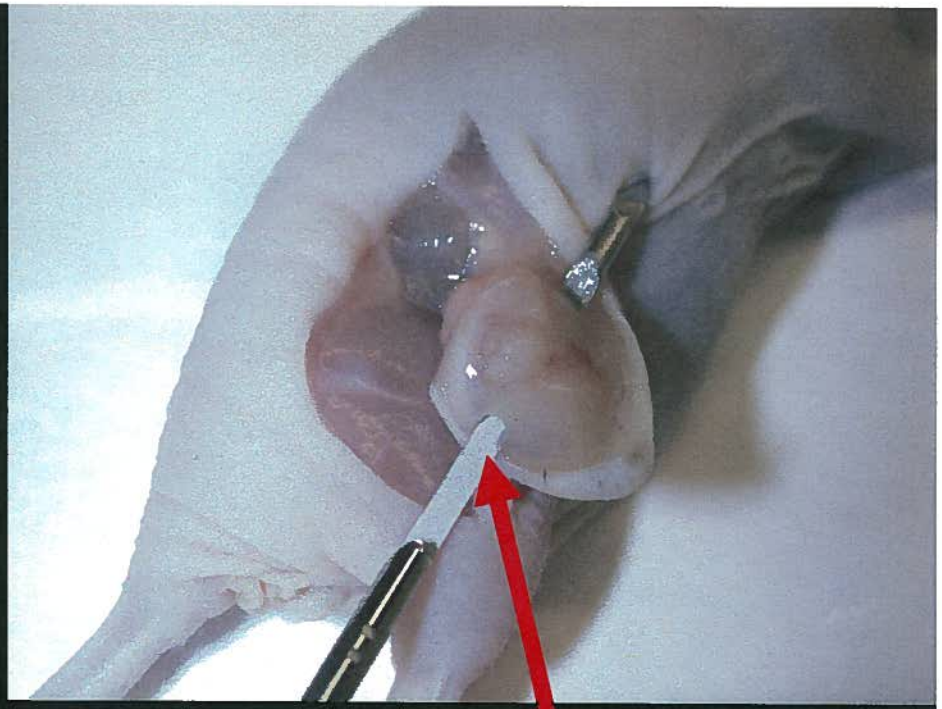
DRUG ANALYSIS

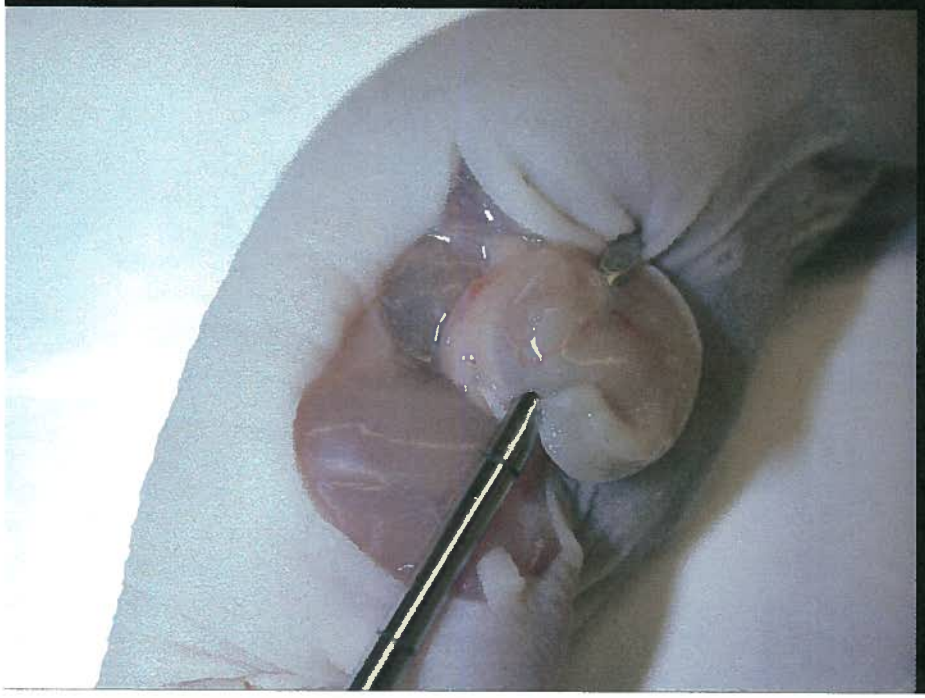
Tools for Analysis

- IHC
  - Original tumor and each passage
  - Antibodies for specific gene products
- Molecular analysis
  - gene expression: microarray (LCM), RT-PCR
  - proteins/phosphoproteins: reverse arrays, IHC
  - biomarkers: serum proteomics
- Analysis of host response
  - immune system response (syngeneic v.s. immunosuppressed)
  - Angiogenesis (MFP v.s. sc)
- Stem Cell search



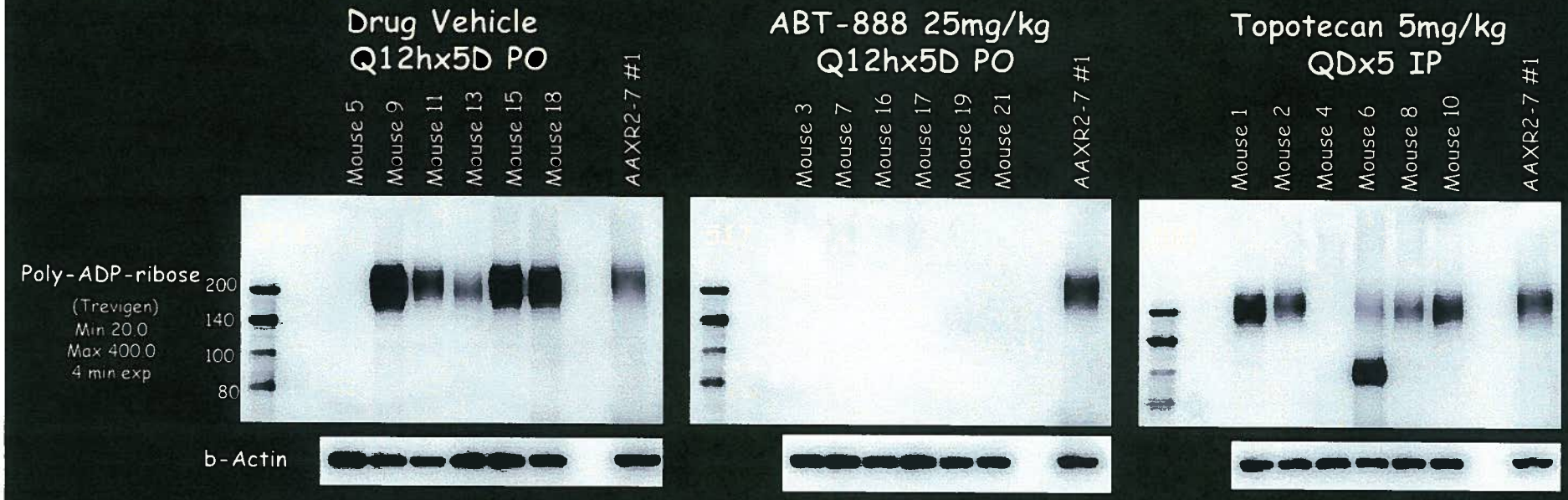




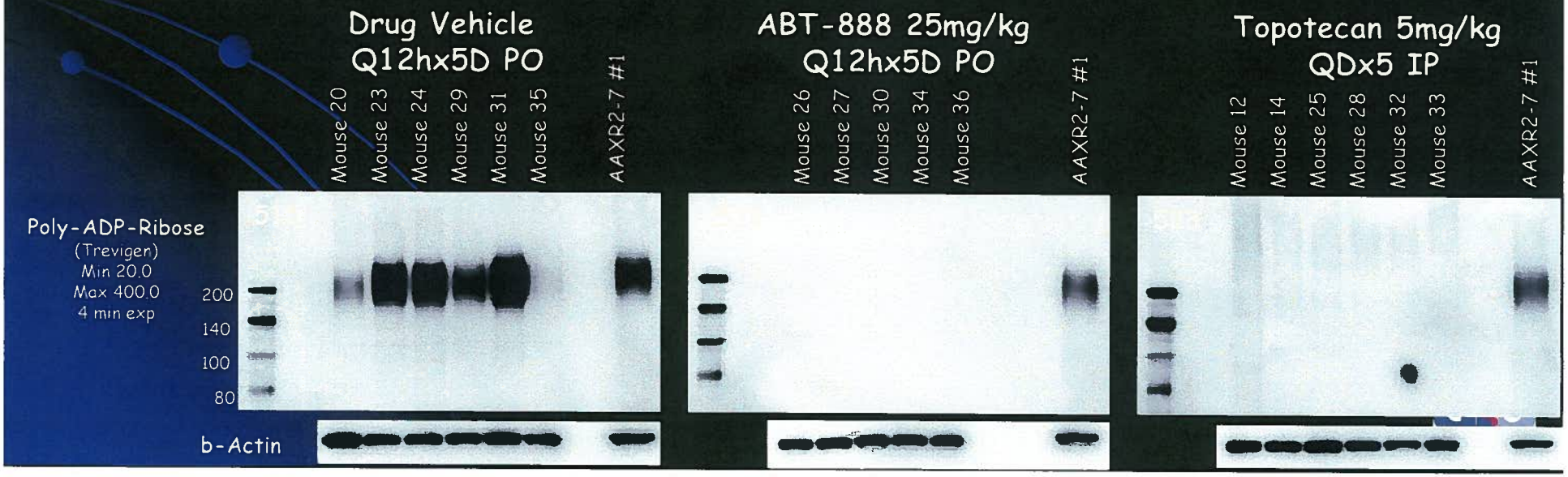


15 ga needle biopsy positioned through subcutaneous tumor with skin reflected so you can actually see how the needle passes through the tumor and exposes the collection area seen in the picture above.

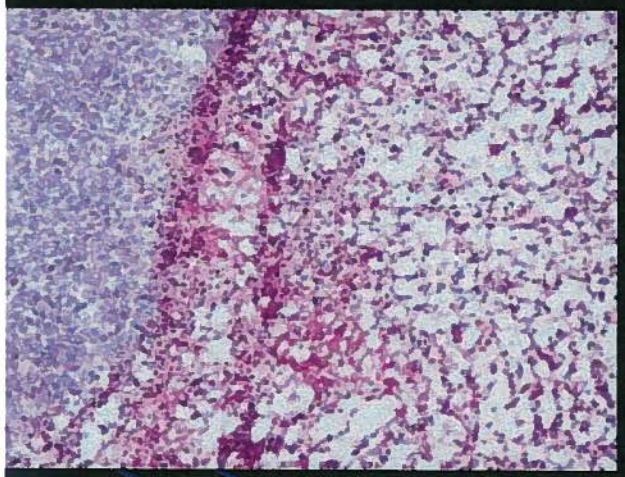
Colo-829 SC Tumors
Collected Post Dose 4

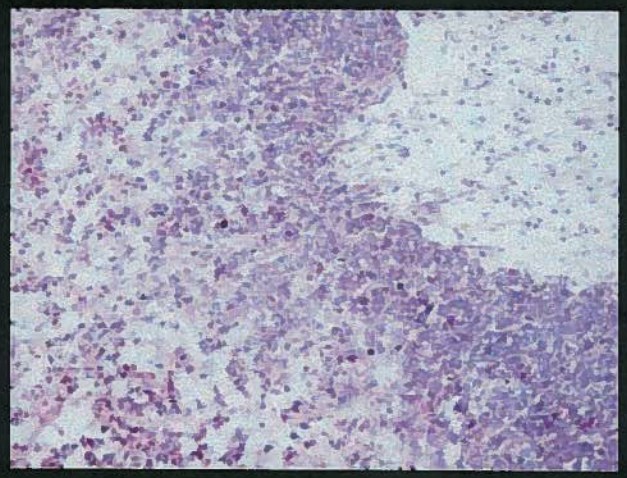


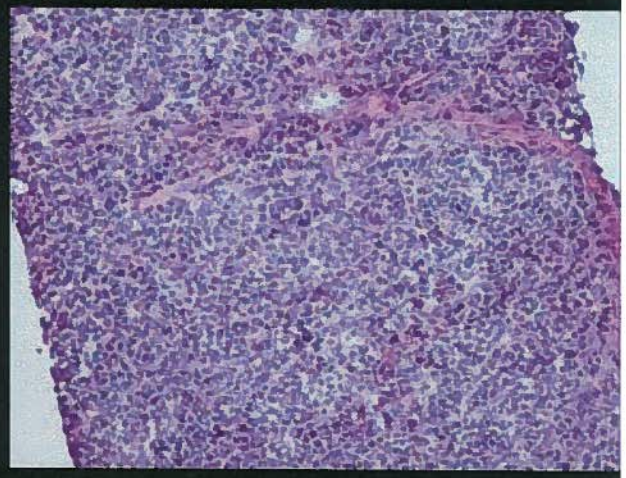
Colo-829 SC Tumors
Collected Post Dose 10



Inconsistency of excisional biopsies







- The first two images show frozen sections of excisional biopsies (H&E).
  - Note the large gaping holes.
- The third image shows a needle biopsy (H&E).

Many Models – Many Options

Statistically valid model assessing relevant endpoints on an optimal schedule with clinically appropriate doses.

With few exceptions, every rodent model, even if conducted with hundreds of experimental mice, represents a single patient. Interpreting the preclinical results and applying these outcomes to human clinical trials continues to prove challenging for those charged with translating the preclinical experience into viable drug candidates.

THANKS!!!

QUESTIONS???