National Cancer Institute

NCI Drug Development Workshop: *How to Advance A Therapeutic Candidate from Bench to Bedside*

Session II. Pre-clinical Proof of Concept: Establishing Activity, Bioavailability, and Associated Effect, in Cancer Relevant Models

July 30, 2021

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
National Institutes of Health

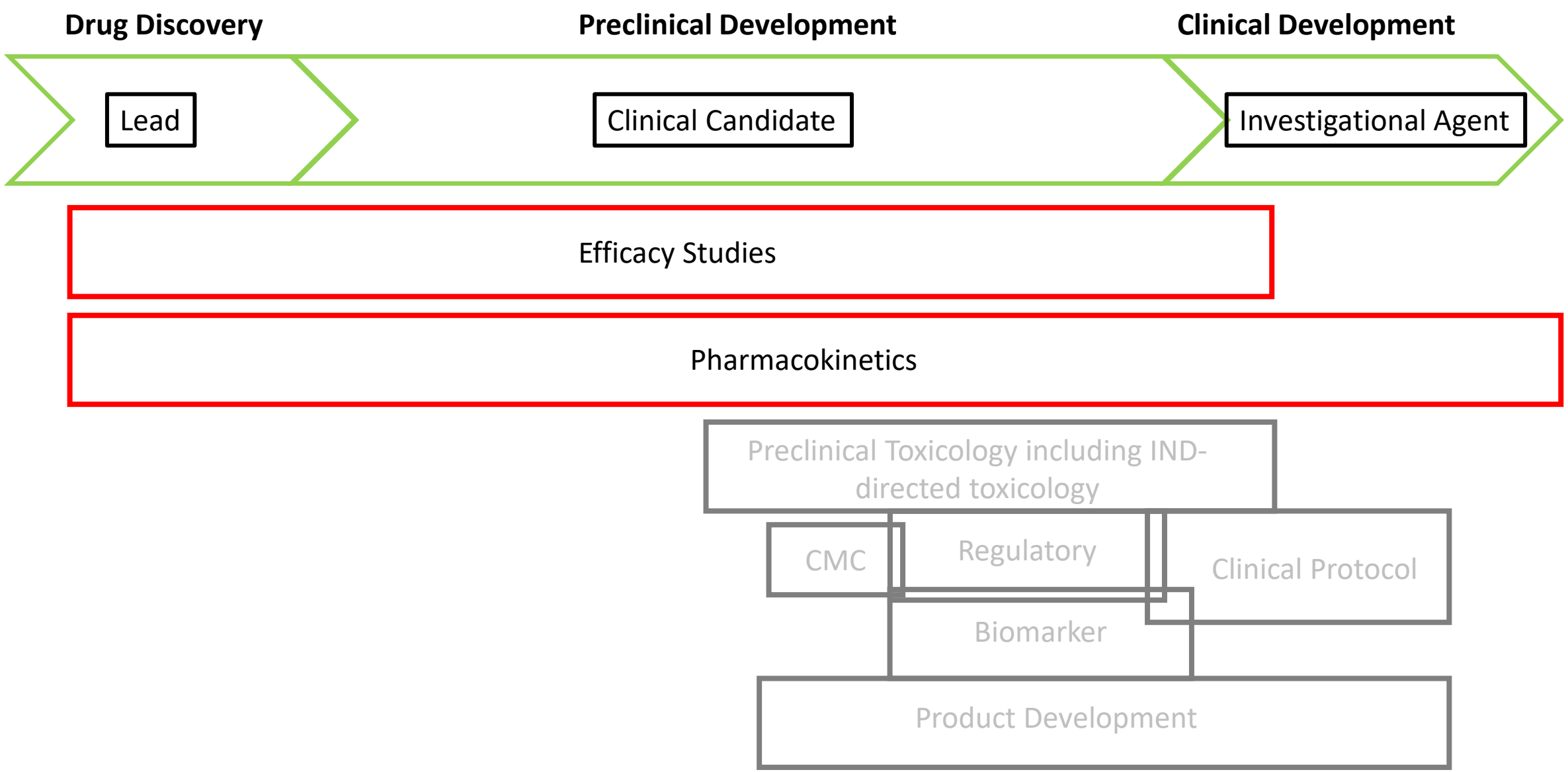
Session II. Pre-clinical Proof of Concept: Establishing Activity, Bioavailability, and Associated Effect, in Cancer Relevant Models

- Topic 1: Establish therapeutic activity of an agent or a combination of agents
  Melinda Hollingshead, D.V.M., Ph.D., National Cancer Institute

- Topic 2: Preclinical pharmacology in IND-enabling studies and clinical pharmacology in clinical protocol development
  Alex Sparreboom, Ph.D., The Ohio State University College of Pharmacy & Comprehensive Cancer Center

NIH NATIONAL CANCER INSTITUTE

July 30, 2021


Drug Discovery
Preclinical Development
Clinical Development
Lead
Clinical Candidate
Investigational Agent
Efficacy Studies
Pharmacokinetics
Preclinical Toxicology including IND-directed toxicology
CMC
Regulatory
Clinical Protocol
Biomarker
Product Development

Establish therapeutic activity of an agent or a combination of agents

Melinda Hollingshead, D.V.M., Ph.D.
Biological Testing Branch
Developmental Therapeutics Program

NIH NATIONAL CANCER INSTITUTE

Why do we use mouse models?

To defend hypotheses regarding potential drug efficacy to justify the costs & risks of clinical trials

- Small, easy to manipulate, many institutions have mouse vivaria
- Does the drug work? (overt tumor growth inhibition and/or target modulation)
- What is happening in the tumor? (PD endpoints)
- How much drug is needed? What's too much? (therapeutic index)
- Will it have efficacy in humans? (rational interpretation of the data and the hypotheses)
- What diseases should be targeted? (mechanism of action?)
- Which molecule is the best in the family? (chemically/structurally related molecules?)

What kinds of rodent models are available?

- **Implanted & Transplanted tumors**
- **Transgenic and knock-out/in tumors**
- **Spontaneous tumors**
  - random, hold mice for lifetime, low incidence
- **Virus-induced tumors**
  - Rauscher, Moloney, LP-BM5, Friend, AKR thymoma, MMTV
- **Carcinogen-induced tumors**
  - Epithelial, GI, Sarcoma, Lung

Implanted & Transplanted Models

These models are commonly used to study diagnostics and interventions.

- **Tissue Source**
  - **Syngeneic (immunocompetent)** - tumor and host are the same inbred strain
  - **Allogeneic (variable)** - same species tumor and host are not fully inbred
  - **Xenogeneic (immunocompromised)** – tumor and host are from different species

- **Implant Site**
  - **Orthotopic** – tumor implanted in tissue matched to origin – e.g., lung into lung
  - **Heterotopic** – tumor implanted into non-matched tissue – e.g., subcutaneous

- **Endpoints** – when is the study complete?

Transplanted Model Characteristics

- Predictable time to tumor occurrence
- Many tumor types available
- Commonly used, historically accepted models
- Do not recapitulate human disease
- Metastatic lesions can be difficult to find

DTP-supported source for human and non-human tumors and cell lines for in vitro and in vivo studies

DTP, DCTD TUMOR REPOSITORY

A CATALOG OF *IN VITRO* CELL LINES, TRANSPLANTABLE ANIMAL AND HUMAN TUMORS AND YEAST

Operated by Charles River Laboratories, Inc.

under contract to the Biological Testing Branch of the National Cancer Institute at Frederick, MD.

Frederick, Maryland 21702-1201

Sponsored by:

Biological Testing Branch
Developmental Therapeutics Program
Division of Cancer Treatment and Diagnosis
National Cancer Institute
National Institutes of Health

DTP Home Page (http://dtp.cancer.gov)

DTP/DCTD/NCI Tumor Repository Request Procedures
Tumor Fragments, Cell Lines and Yeast

The DCTD Tumor Repository is currently updating the Web Site and the request procedures and forms.

To request information and forms on how to place a request please send an email to DCTDTumorRepository@mail.nih.gov

Costs for cell and tumor lines.

Cell or Tumor Lines	NCI/NIH Investigators & Fed. Govt. (MD campuses only)	Academia & Non-Profits both Domestic & International	Commercial Entities both Domestic & International
Per Cryopreserved Vial	N/A	$150.00	$150.00
NCI Anti-Cancer Cell Line Panel	N/A	$8,850.00	$6,900.00

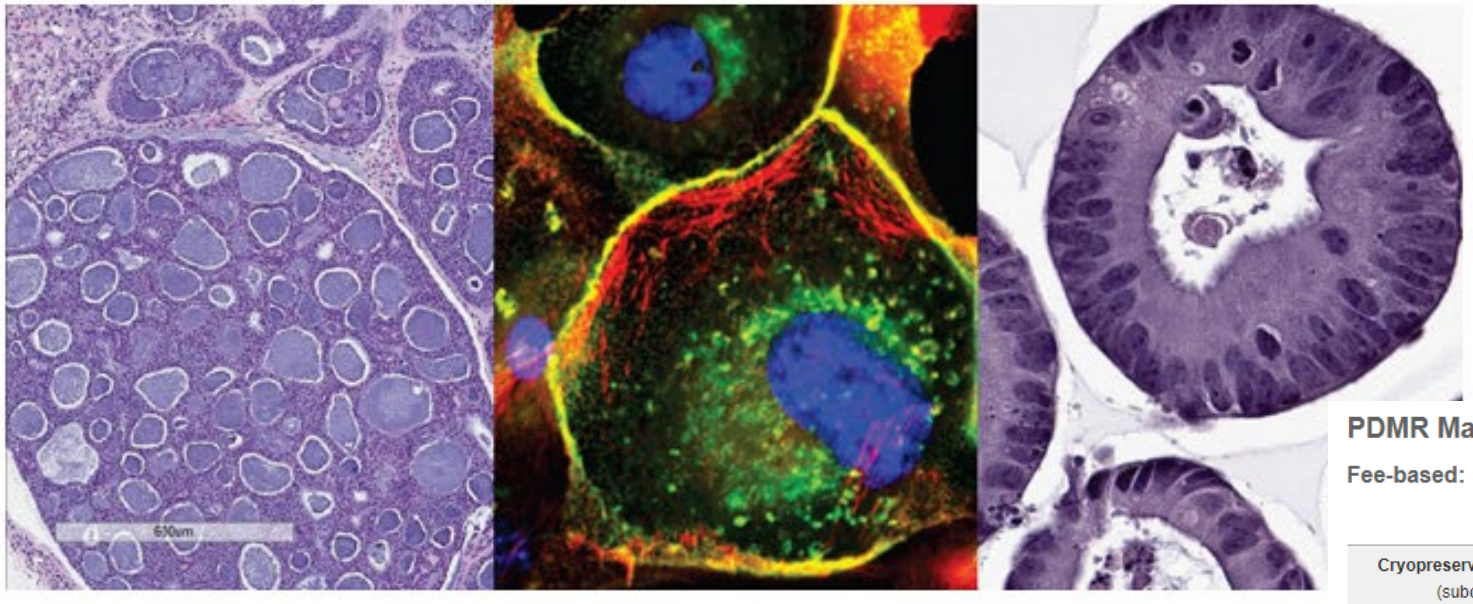
https://dtp.cancer.gov/organization/btb/docs/DCTDTumorRepositoryCatalog.pdf

PDX – Patient Derived Xenografts

- Direct implantation of patient tumor material with serial passage through mice
- Grown in immunocompromised mice like other xenogeneic models
- Time to tumor occurrence can be protracted
- More tumor heterogeneity than cell line xenografts

Home | About the PDMR | PDMR Models | PDMR Database | SOPs | Publications | Requesting Material | Citing PDMR | Contact Us

NCI Patient-Derived Models Repository (PDMR)



About the PDMR

- How to Request PDMR Material
- PDMR Database
- Contact Us

PDMR Materials Available

Fee-based: Submission Request Required

In Vivo	In Vitro
Cryopreserved Patient-Derived Xenograft (PDX) Fragments (subcutaneous implantation into 2-5 NSG mice)	**Patient/PDX-Derived heterogenous tumor culture cells (PDC)** (in vitro culture, defined media, tested for growth as a cell line xenograft in NSG mice, minimum of 7.5 x 10^5 cells)
RNA from fresh-frozen PDX tissue (2-3 µg in at least 10 µL)	**Patient/PDX-Derived organoids (PDOrg)** (3D in vitro culture, defined media, tested for growth as a cell line xenograft in NSG mice, minimum of 7.5 x 10^5 cells)
DNA from fresh-frozen PDX tissue (2-3 µg in at least 10 µL)	**Cancer-Associated Fibroblasts (CAF)** (limited lifespan, not transformed, defined media, minimum of 1-5 x 10^5 cells)
Fresh-Frozen PDX fragments (30-60 mg; protein/nucleotide extraction)	

Publicly Accessible Genomics Data: Download from PDMR Database

Note: Data are generated from PDX tumors which are a mixture of human tumor and mouse stroma; these are not pure human extractions. Murine reads are removed bioinformatically. Data analysis pipelines and reference sequence for the NSG host (NOD.*Cg-Prkdc*^{scid} *Il2rg*^{tm1Wjl}/SzJ) can be found on the PDMR SOP page.

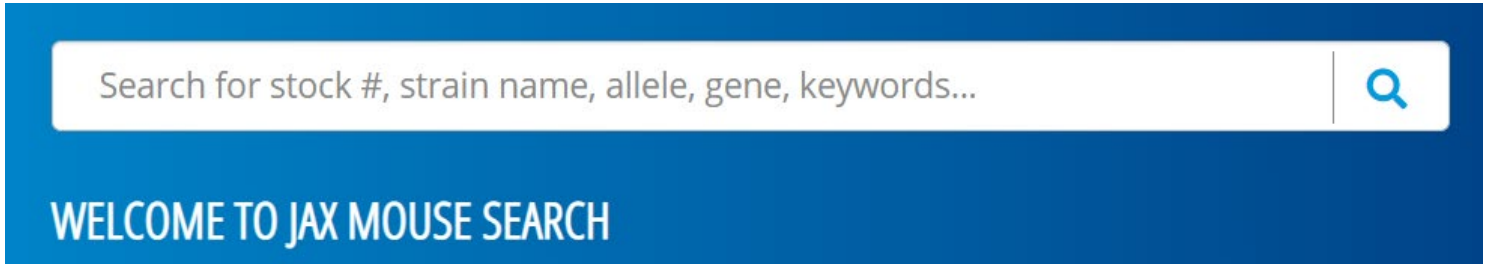
In Vivo (PDX)	In Vitro (PDC and PDOrg only)

https://pdmr.cancer.gov/

Genetically altered Models

cancer genetics, cancer progression and therapeutics testing

- **Many available through commercial and collaborative arrangements**
  - **http://mouse.ncifcrf.gov/**
  - **http://jaxmice.jax.org/query/f?p=205:1:15102992284346591 65**
- **May be patented**
- **Tumor incidence may be low**
- **Tumor latency may be protracted**
- **Breeding schemes may be complex**
- **Well-characterized genetic alterations**
- **Disease may follow a more natural course**
- **Resulting tumors may be transplantable**



What if mice are not appropriate for your studies – what are the options?

Non-murine models requiring only small animal vivaria

- Rat tumors – Fischer 344, Buffalo, Wistar, Sprague-Dawley, Noble
- Hamster tumors – golden Syrian host
- Rabbit tumors – Brown-Pierce tumor, VX2 tumor - used for eye, liver and imaging studies

See https://www.frontiersin.org/research-topics/7694/humanized-large-animal-cancer-models-accelerating-time-and-effectiveness-of-clinical-trials#articles for a discussion of the large animal models being applied to cancer including pigs, sheep, dogs

Porcine models:

- SCID pig
- Transplantable tumors in inbred pigs
- Transgenic pigs

Ovine spontaneous pulmonary adenocarcinoma

NCI CCR Comparative Oncology Program studies cancers in pet dogs and sponsors clinical trials in dogs
https://ccr.cancer.gov/Comparative-Oncology-Program

NCI-Funded Canine Immunotherapy Trials Network Treats Pet Dogs to Study Cancers Common to Humans
https://dctd.cancer.gov/NewsEvents/20190327_canine_immunotherapy.htm

Integrated Canine Data Commons (ICDC)
https://datacommons.cancer.gov/repository/integrated-canine-data-commons#:~:text=The%20Integrated%20Canine%20Data%20Commons,comparative%20analysis%20with%20canine%20cancer.&text=Canines%20are%20also%20of%20scientific,to%20the%20ICDC%20data%20model

The appropriateness of animal models to identify, qualify and promote new therapies for cancer has been under review, and in some ways under attack, for many years. Continuing concerns about the failure rate of agents being sent to the clinic has led to a flurry of publications on the irreproducibility of published preclinical data and their over-prediction of activity.

Opinionator
Exclusive Online Commentary From The Times

May 2, 2011, 9:15 PM

Helping New Drugs Out of Research's 'Valley of Death'

By DAVID BORNSTEIN

Fixes looks at solutions to social problems and why they work.

TAGS:

DRUGS (PHARMACEUTICALS), MEDICINE AND HEALTH, MULTIPLE SCLEROSIS, RESEARCH

Consider two numbers: 800,000 and 21.

The first is the number of medical research papers that were published in 2008. The second is the number of new drugs that were approved by the Food and Drug Administration last year.

That's an ocean of research producing treatments by the drop. Indeed, in recent decades, one of the most sobering realities in the field of biomedical research has been the fact that, despite significant increases in funding — as well as extraordinary advances in things like genomics, computerized molecular modeling, and drug screening and synthesization — the number of new treatments for illnesses that make it to market each year has flatlined (pdf) at historically low levels.

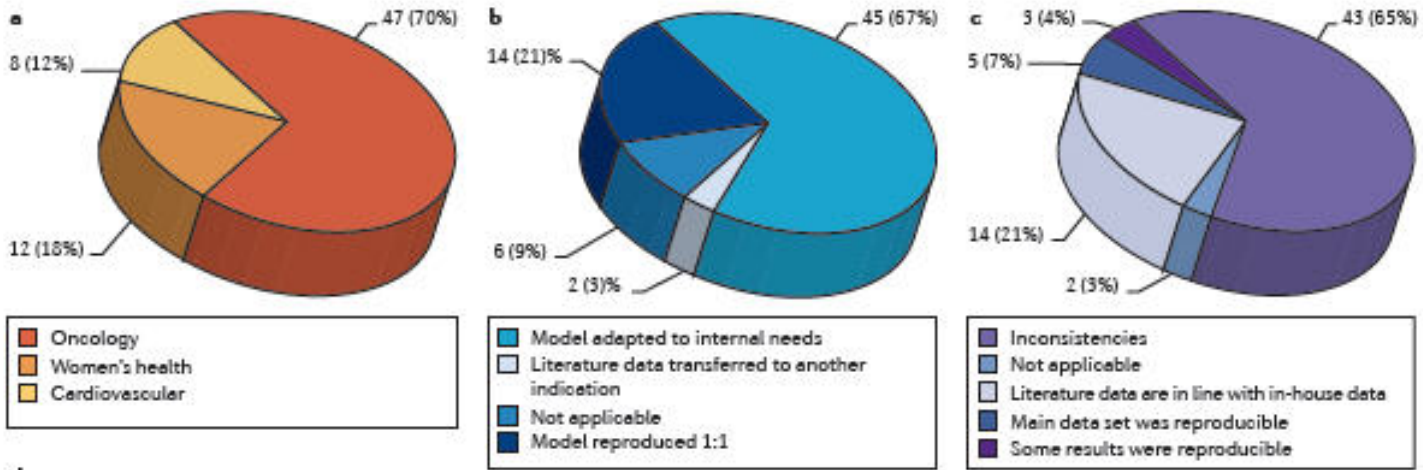
An ocean of research is producing cures and treatments by the drop.

LINK TO ORIGINAL ARTICLE

Believe it or not: how much can we rely on published data on potential drug targets?

Florian Prinz, Thomas Schlange and Khusru Asadullah

results that are published are hard to reproduce. However, there is an imbalance between this apparently widespread impression and its public recognition (for example, see REFS 2,3), and the surprisingly few scientific publications dealing with this topic. Indeed, to our knowledge, so far there has been no published in-depth, systematic analysis that compares reproduced results with published results for wet-lab experiments related to target identification and validation.



d

	Model reproduced 1:1	Model adapted to internal needs (cell line, assays)	Literature data transferred to another indication	Not applicable
In-house data in line with published results	1 (7%)	12 (86%)	0	1 (7%)
Inconsistencies that led to project termination	11 (26%)	26 (60%)	2 (5%)	4 (9%)

Figure 1 | **Analysis of the reproducibility of published data in 67 in-house projects. a** | This figure illustrates the distribution of projects within the oncology, women's health and cardiovascular indications that were analysed in this study. **b** | Several approaches were used to reproduce the published data. Models were either exactly copied, adapted to internal needs (for example, using other cell lines than those published, other assays and so on) or the published data was transferred to models for another indication. 'Not applicable' refers to projects in which general hypotheses could not be verified. **c** | Relationship of published data to in-house data. The proportion of each of the following outcomes is shown: data were completely in line with published data; the main set was reproducible; some results (including the most relevant hypothesis) were reproducible; or the data showed inconsistencies that led to project termination. 'Not applicable' refers to projects that were almost exclusively based on in-house data, such as gene expression analysis. The number of projects and the percentage of projects within this study (**a**– **c**) are indicated. **d** | A comparison of model usage in the reproducible and irreproducible projects is shown. The respective numbers of projects and the percentages of the groups are indicated.

Friday, December 2, 2011 As of 12:00 AM New York 68°|55°

THE WALL STREET JOURNAL. | HEALTH

GET F SUBSC

U.S. Edition Home • Today's Paper • People In The News • Video • Blogs • Journal Community

World • U.S. • New York • Business • Markets • Tech • Personal Finance • Life & Culture • Opinio

Asia Europe Earnings Economy Health Law Autos Management Media & Marketing Energy CF

TOP STORIES IN Business

1 of 12 Oprah Struggles to Build Her Network

2 of 12 Lenovo Reaches Beyond PCs

FDA for Pr

HEALTH INDUSTRY | December 2, 2011

Scientists' Elusive Goal: Reproducing Study Results

Article | Video | Stock Quotes | Comments (74)

Save | Recommend 507 | Send | Tweet 269 | A A

ARTICLE FREE PASS

Enjoy your free sample of exclusive subscriber content.

GET ALL OF WSJ.COM: SUBSCRIBE NOW - GET 8 WEEKS FREE

By GAUTAM NAIK

Two years ago, a group of Boston researchers published a study describing how they had destroyed cancer tumors by targeting a protein called STK33. Scientists at biotechnology firm Amgen Inc. AMGN -0.50% quickly pounced on the idea and assigned two dozen researchers to try to repeat the experiment with a goal of turning the findings into a drug.

WSJ's Gautam Naik has details of challenges

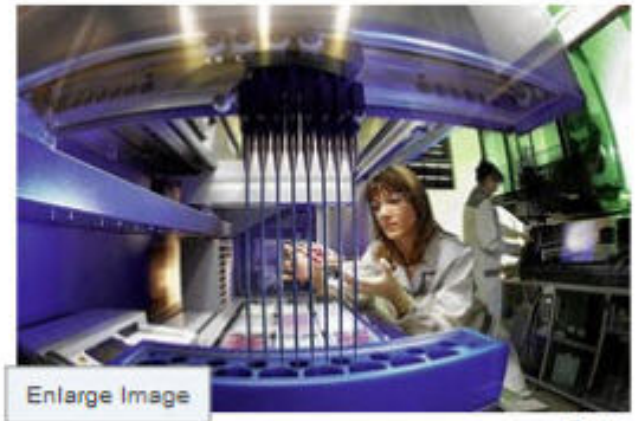
It proved to be a waste of time and money. After six months of intensive lab work, Amgen found it couldn't replicate the results and scrapped the project.

"I was disappointed but not surprised," says Glenn Begley, vice president of research at Amgen of Thousand Oaks,

WSJ's Gautam Naik has details of challenges scientists face in reproducing claims made by medical journals. Photo: Sandy Huffaker/The New York Times

"I was disappointed but not surprised," says Glenn Begley, vice president of research at Amgen of Thousand Oaks, Calif. "More often than not, we are unable to reproduce findings" published by researchers in journals.

This is one of medicine's dirty secrets: Most results, including those that appear in top-flight peer-reviewed journals, can't be reproduced.



Enlarge Image

Bayer

Researchers at Bayer's labs often find their experiments fail to match claims made in the scientific literature.

"It's a very serious and disturbing issue because it obviously misleads people" who implicitly trust findings published in a respected peer-reviewed journal, says Bruce Alberts, editor of Science. On Friday, the U.S. journal is devoting a large chunk of its Dec. 2 issue to the problem of scientific replication.

Reproducibility is the foundation of all modern research, the standard by which scientific claims are evaluated. In the U.S. alone, biomedical research is a $100-billion-year enterprise. So when published medical findings can't be validated by others, there are major consequences.

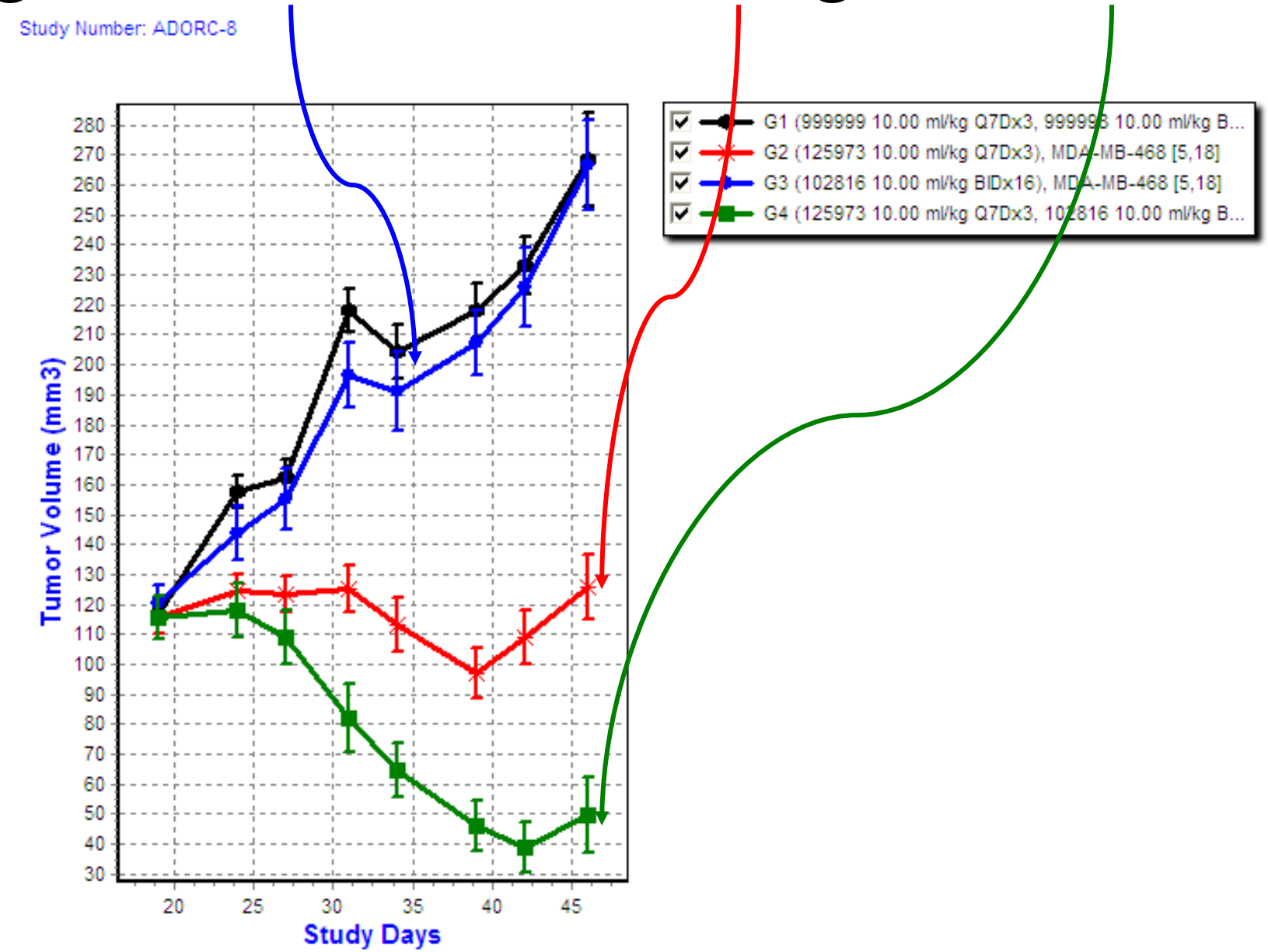
What can you do?

- Use well powered animal studies [n= 3 to draw final conclusions is not adequate]
- Reproduce your own data
- Have 2 separate operators generate the data
- Provide adequate details in publications for others to replicate
- Don’t over-interpret your data
- Stage tumor studies correctly
- Don’t selectively use/present your data
- Remember the clinical situation and what can be assessed in man

Common Clinical Endpoints In Man

- **Toxicity**
- **Tumor response**
  - Biomarker modulation as a measure of the effect of a treatment that may correlate with a traditional clinical endpoint (PFS; TR)
  - Progression-free survival (stable disease)
  - Tumor regression
- **Survival**
- **Quality of life**

Progression, Stable, Regression



In Vivo Efficacy Models

- **Human Tumors**
  - **Subcutaneous**
  - **Intravenous**
  - **Intraperitoneal**
  - **Orthotopic**
    - **Mammary fat pad**
    - **Intracranial**
    - **Intrarenal**
    - **Intrahepatic**
    - **Intracecal**
    - **Intracranial**
    - **Intrapancreatic**
    - **intraprostatic**

- **Rodent Tumors**
  - **Subcutaneous**
  - **Intravenous**
  - **Intraperitoneal**
  - **Orthotopic**
  - **Metastatic**
  - **Transgenic**
  - **Knock-in/out**

- **Tumor sources**
  - **Cells cultured in vitro**
  - **Serially passaged tumor**
  - **Cryopreserved tumor**

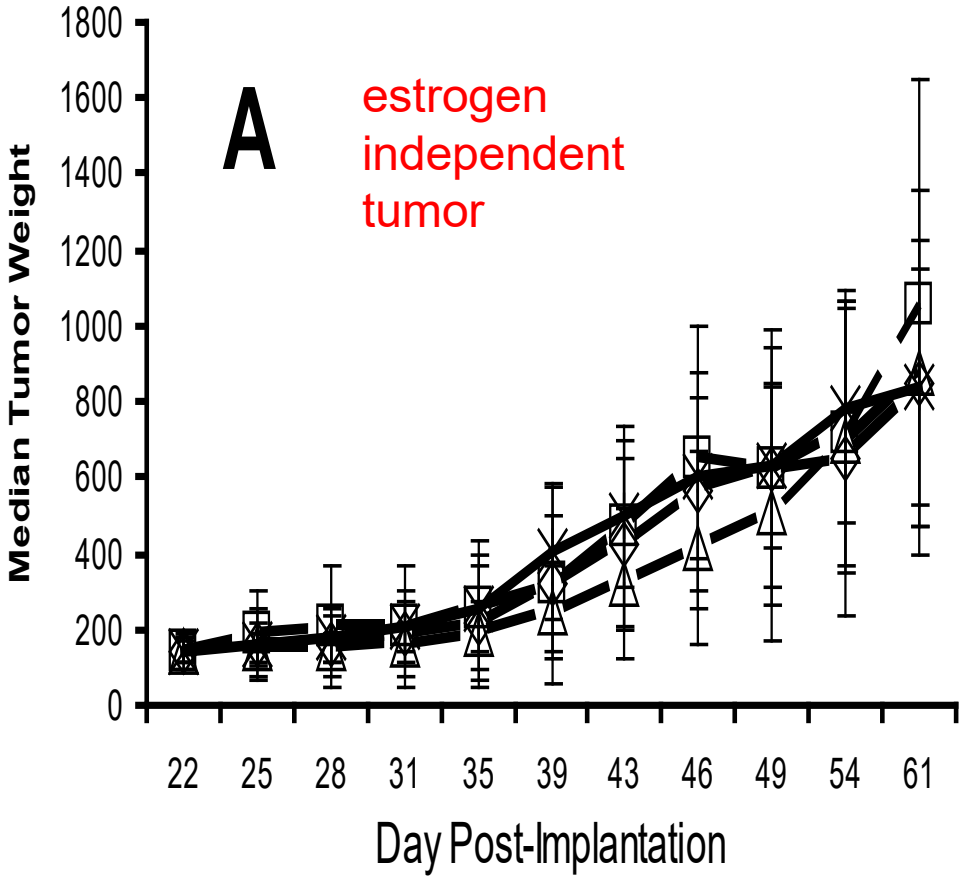
Questions in Efficacy Evaluations

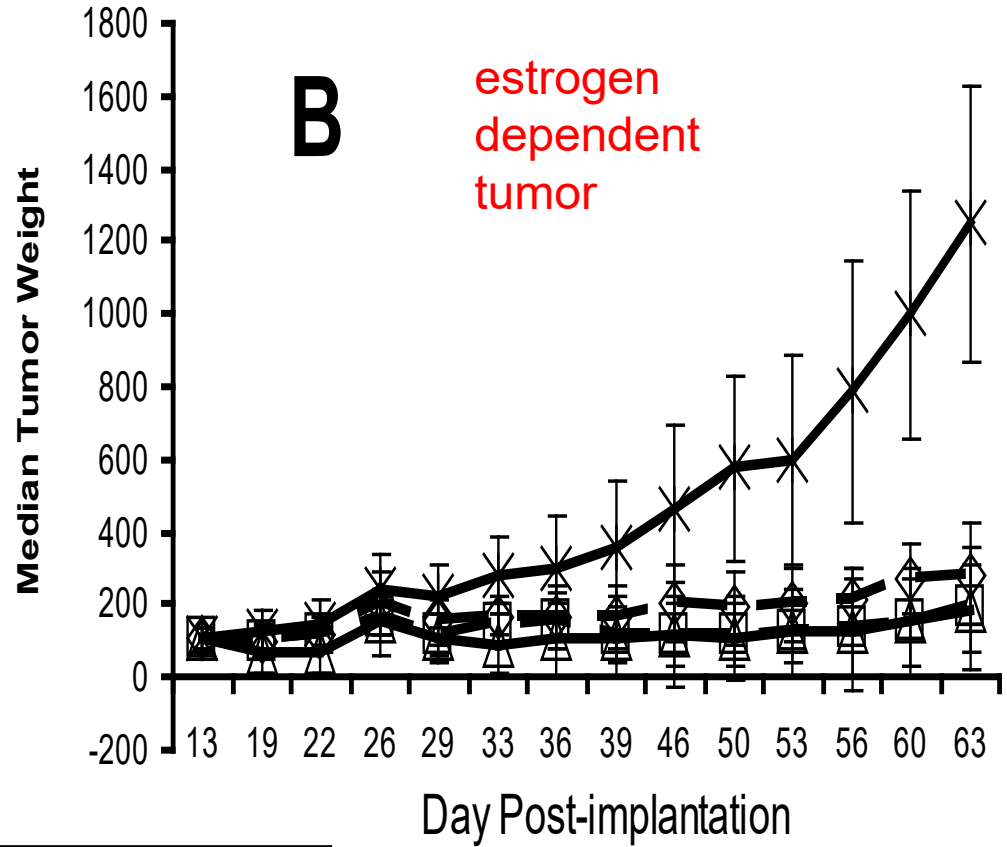
- Which model(s)
- Dose, route and schedule
- Vehicle, formulation, stability
- Experimental protocol
- Pharmacologic and pharmacokinetic readouts
- Endpoints

Efficacy Model Selection

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

Efficacy Model Selection





The "drug" target must be present AND required for continued growth of the tumor

100% sesame oil
45 mg/kg tamoxifen
22.5 mg/kg tamoxifen
11.25 mg/kg tamoxifen

See:

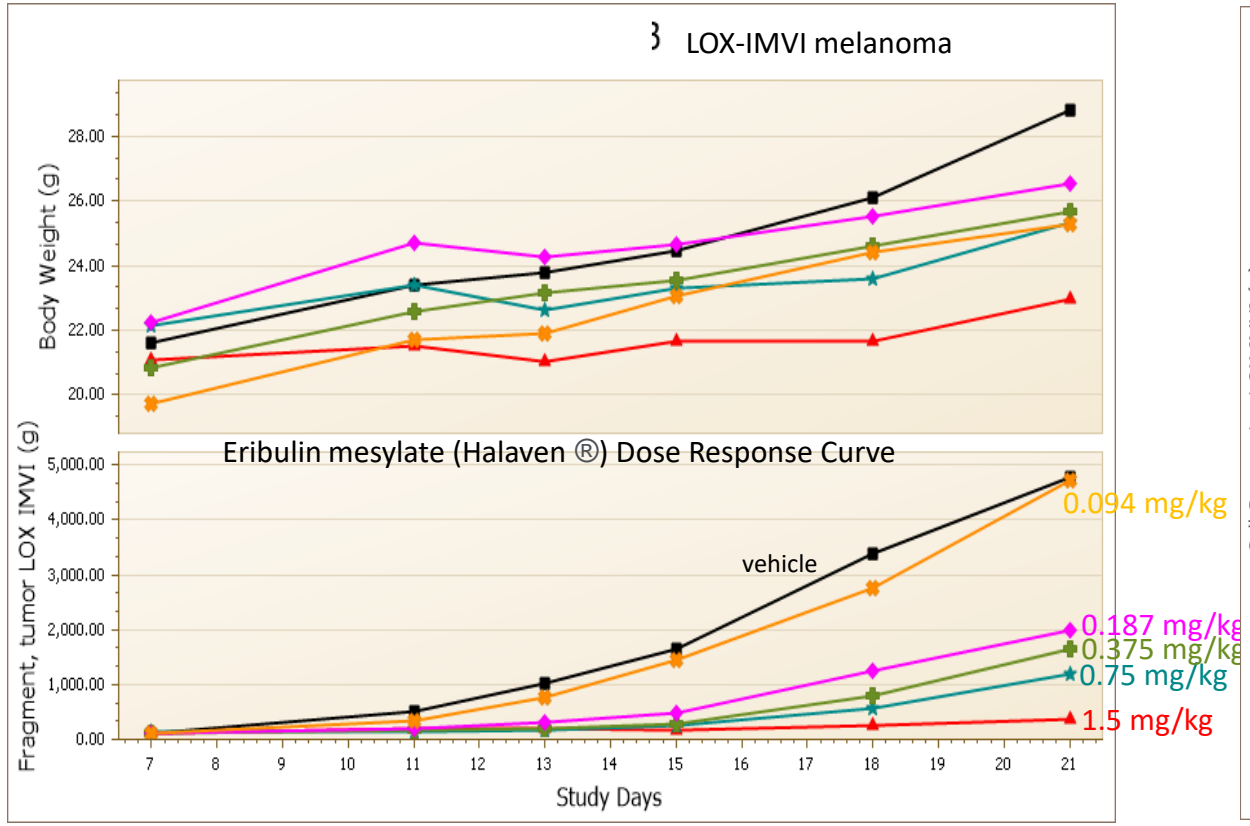
https://doi.org/10.1093/jnci/djn351

Dose, Route, Schedule

- Published/prior knowledge?
- Proposed/expected mechanism?
- How much exposure is required for effect? Continuous? Intermittent?
- Is the material soluble/stable in aqueous solution and compatible with mice?
- What routes of administration are technically feasible?
- Options
  - What are the maximum tolerated dose (MTD) and the Minimally effective dose (MED)?
  - Typical routes - IP, IV, SC, PO
  - Dose schedule: QDx?; Q2Dx?, Q3Dx?, BIDx?; TIDx?
- For combinations – how much can be given? Will schedule be critical – A+B, A then B, B then A?

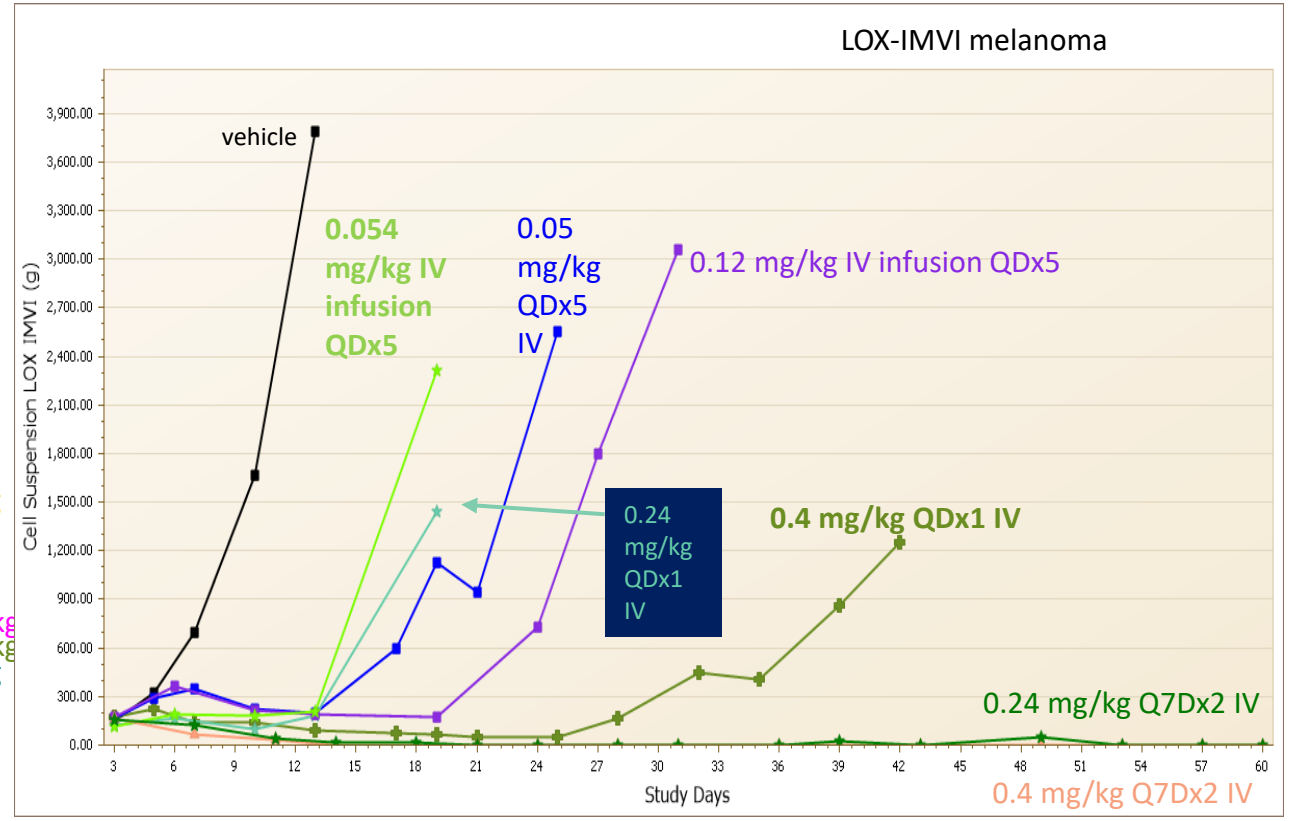
What is nice to see when evaluating efficacy data

A dose response curve



All administrations were bolus IV doses given via the tail vein on a Q4Dx3 schedule. Vehicle was 2% Ethanol in 0.9% saline. Dose volume: 0.1 ml/10gm BW

A dosing schedule comparison

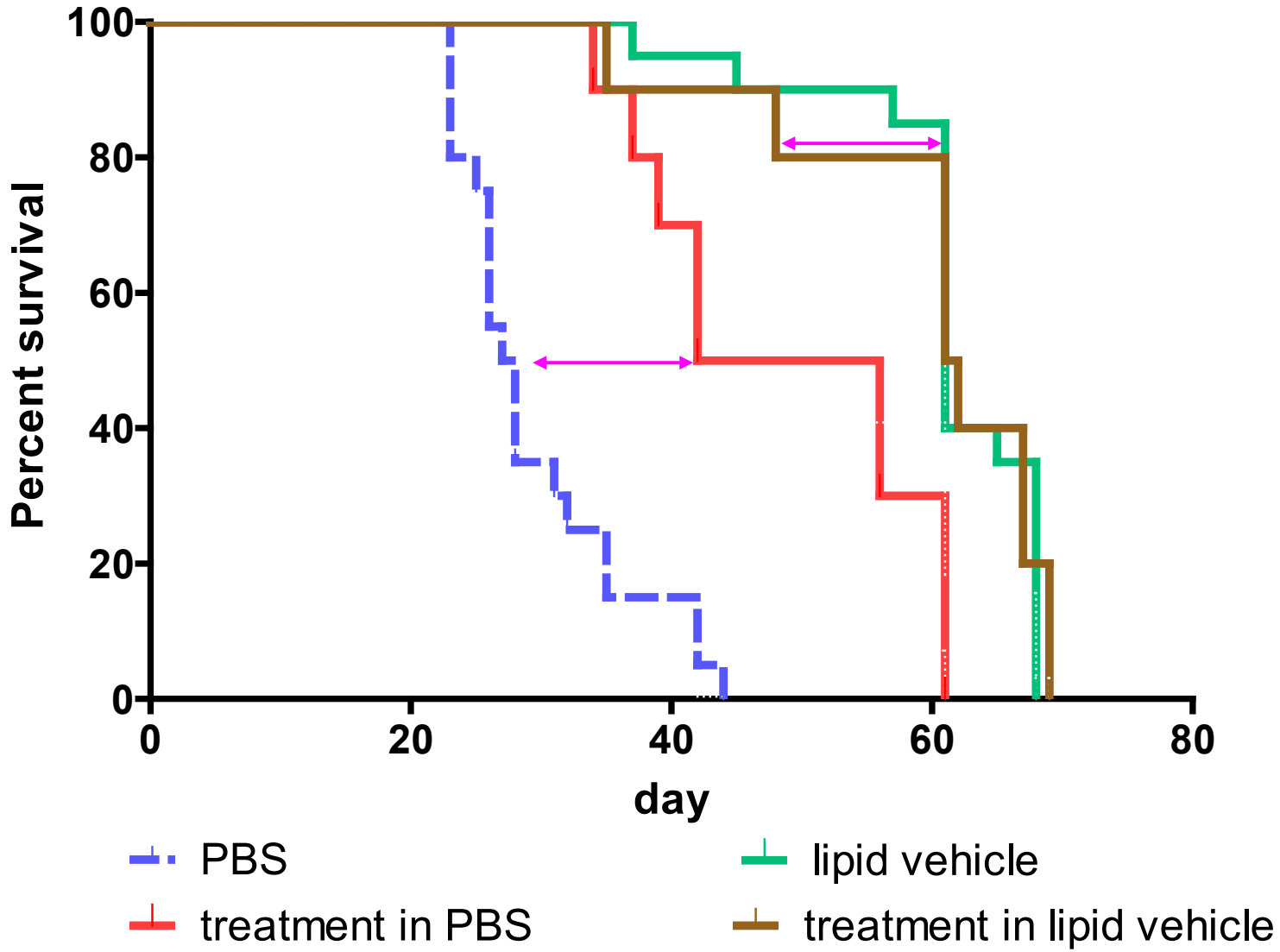


5 day infusions were achieved using SC implanted osmotic pumps connected to indwelling jugular catheters. All other administrations were bolus IV doses given via the tail vein. Vehicle was 1% ethanol in 0.9% saline with 0.05% Tween 80. Dose volume: 0.1 ml/10gm BW for bolus, volume for osmotic pumps was 1 ul/hr using an Alzet® Model 2001 pump.

Vehicle, formulation, stability for preclinical studies

- Definitive clinical vehicle and formulation not required
- KISS - Use the simplest vehicle that works
- Vehicle tolerability– e.g., PEG given PO can cause diarrhea; DMSO is tolerated @ 3uL/gm
- Determine stability in solution or prep fresh solutions for each dose
- Consider a 100% DMSO stock solution, aliquot, freeze and dilute for dosing
- 0.9% saline (physiological saline) and D5W (5% dextrose in water) are physiological dosing solutions PBS is not a physiological buffer
- Consider a 100% ethanol stock solution, aliquot, freeze and dilute with D5W or saline to dose. Insoluble compounds may work in ethanol:cremophor:D5W (10:10:80)
- Include a vehicle control in ALL studies not just an untreated control

Vehicle selection and experimental design are important to the outcome



See:

https://doi.org/10.1093/jnci/djn351

Kaplan-Meier Plot of mice bearing intraperitoneal OVCAR-5 human ovarian cancer xenografts. Mice were treated with vehicle (PBS or lipid vehicle) or with a therapeutic solubilized in each of the vehicles. Note the lipid vehicle alone was as effective in improving survival as was the therapeutic prepared in the lipid vehicle and it was more effective than the therapeutic prepared in PBS.

Experimental Protocol

- When will treatment start? early vs advanced
- When will treatment end?
- How will the animals be randomized?
- Will samples be collected for ex vivo evaluation?
- Will tumors be monitored visually? By imaging techniques? By take-down timepoints?
- What will terminate the experiment, i.e., what are the humane endpoints?

Staging Tumors

- Implant more mice than the study requires so you can select a range of mice to randomize into the study. The percent excess will depend upon the take rate and heterogeneity in growth rates for the tumor model

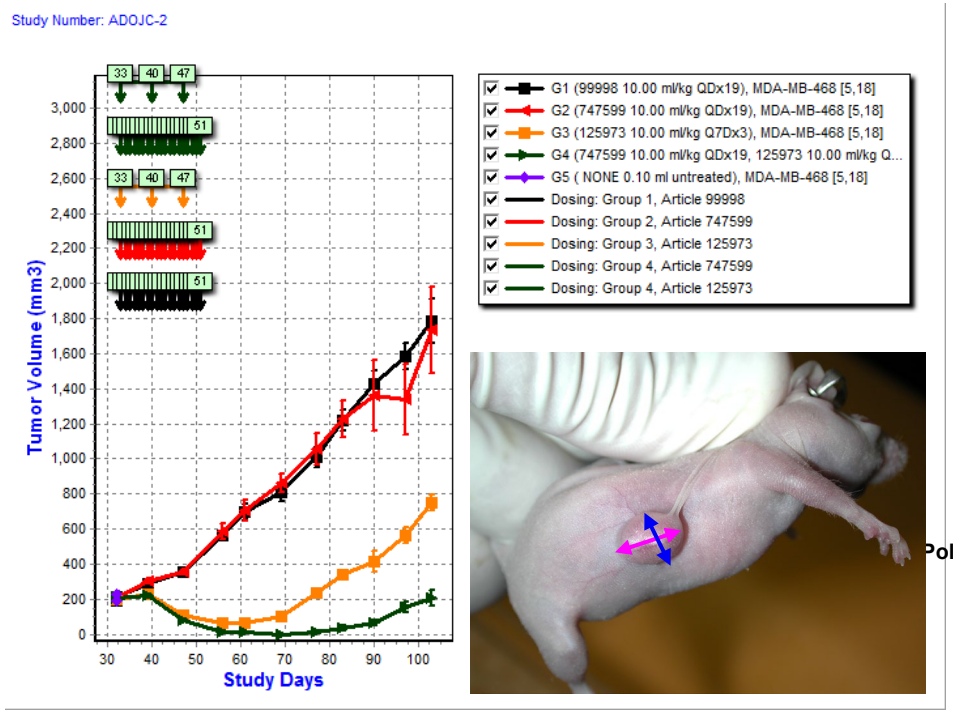
- As tumors grow monitor growth until a cohort of tumors reach the size range desired
  - early stage treatment – tumors staged between 75-225 mg
  - advanced stage treatment – tumors staged between 200-400 mg

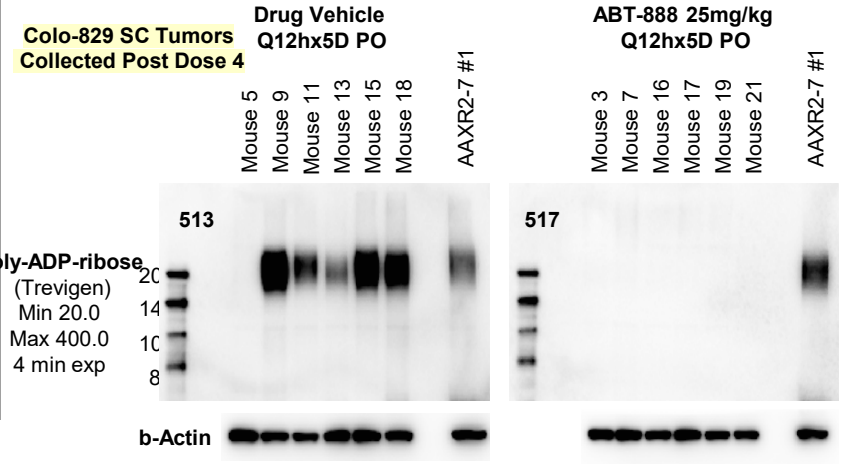
- Randomize the mice into treatment groups. For manual randomization we use a ranked randomization method

Endpoints

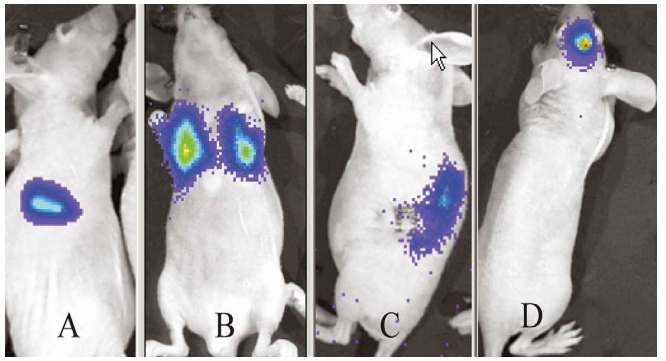
- Tumor size
- Weight loss (less than 10% is desired)
- Time to sacrifice
- Imaging
- Pre-defined time of termination
- Time post-treatment – remember holding the mice beyond last treatment day shows durability of effect

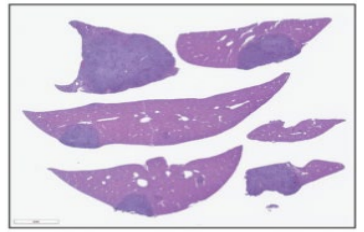
Monitoring drug effect

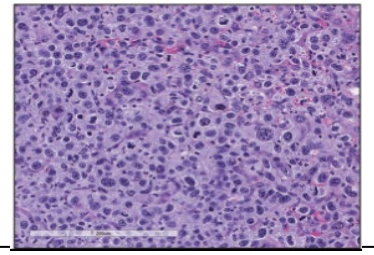




Bioluminescent imaging of tumors

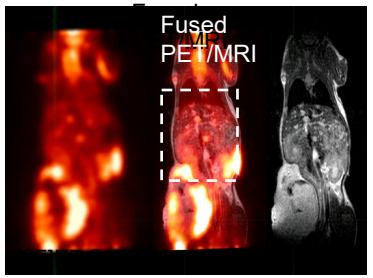






Liver H&E,
Multifocal metastatic lesions

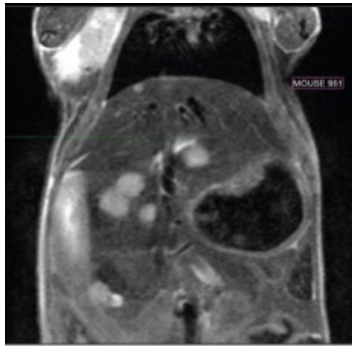
MRI/PET Fusion: enhancement for metabolic metastatic analysis

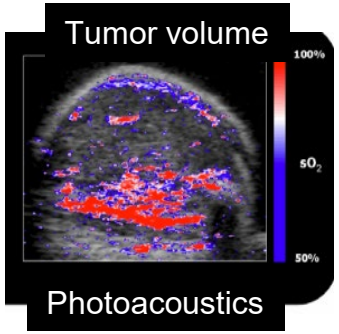


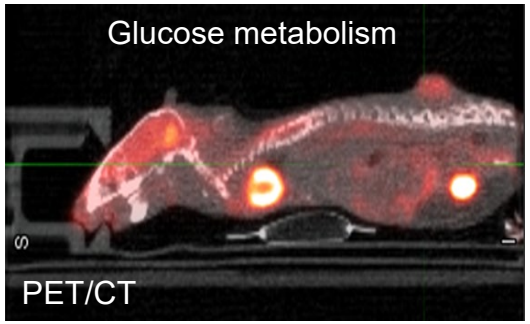
[^{18}F]FDG PET

T2w 1T MRI

Anatomical MRI



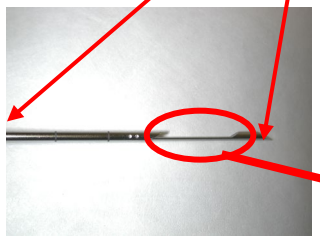


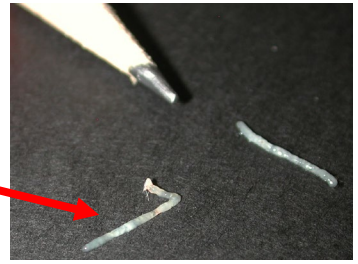


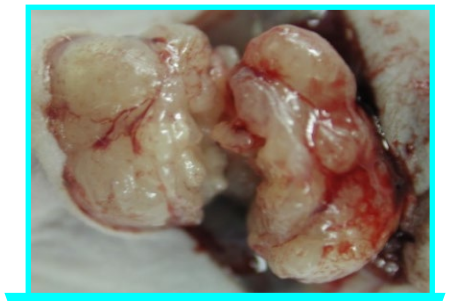
Possibilities with In Vivo Studies

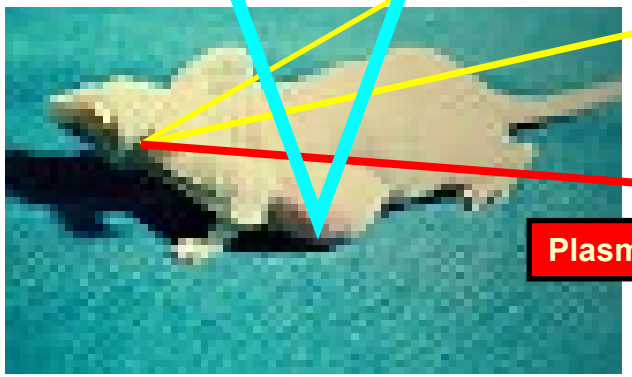
Each tumor can provide multiple endpoint readouts

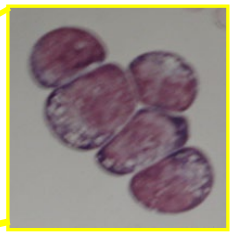
18 ga Temno® biopsy needle











PK

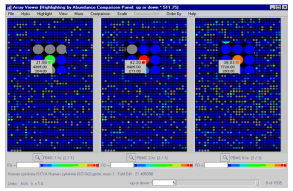
IHC

Harvest and flash freeze

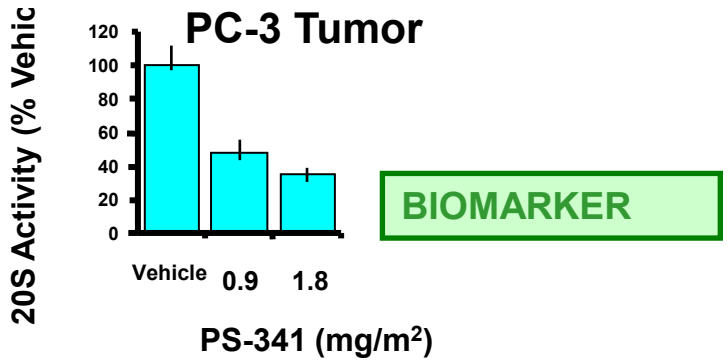
Snap-Frozen (RNA)

Plasma

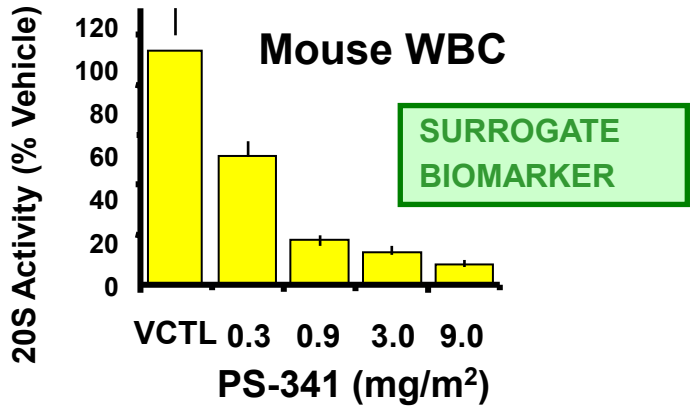
Tumor RNA



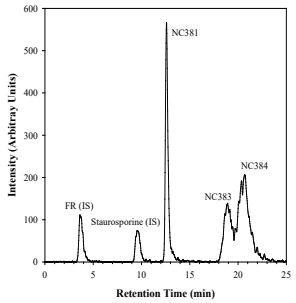
GENOMICS



BIOMARKER

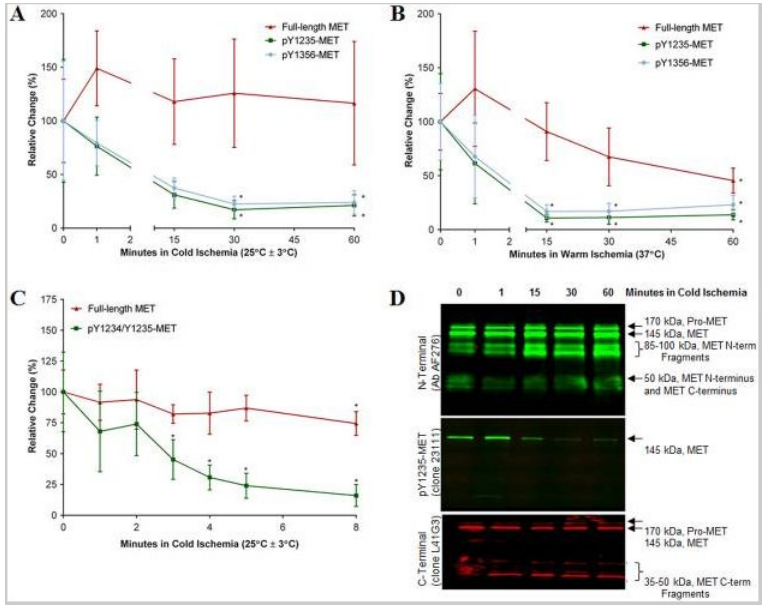


SURROGATE BIOMARKER



DRUG ANALYSIS

Understand your target's stability

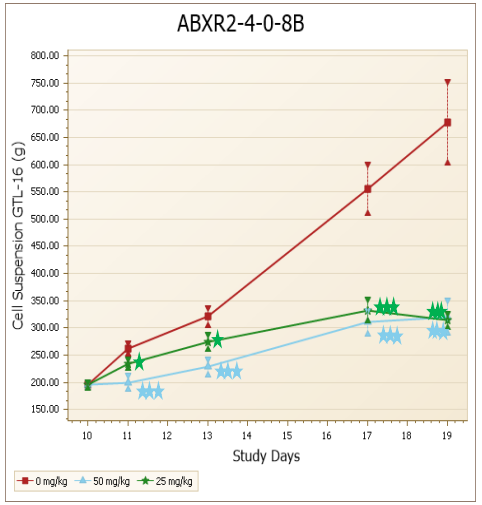


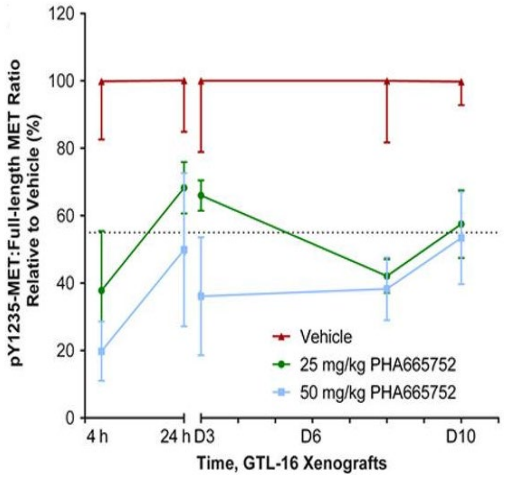
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7802886/

Stability of full-length MET and pMET in core needle biopsies of xenografted SNU5 tumors.

Core needle biopsies were incubated in saline solution for 0 (baseline, 100%), 1, 15, 30, or 60 min of **(A)** cold ischemia or **(B)** warm ischemia. **(C)** Follow-up study of stability of full-length and pY1234/1235-MET in core biopsies during 0, 1, 2, 3, 4, 5, and 8 min of cold ischemia. For all graphs, error bars are mean ± SD, $n = 4$-6; a single asterisk (*) denotes $P < 0.05$ from baseline by Student's t test. **(D)** Western blot of extracts of core biopsies after 0, 1, 15, 30 or 60 min of cold ischemia probed with the indicated antibodies.

More drug is not necessarily better: c-met kinase inhibitor PHA665752 was to be dosed intraperitoneally for 10 days at 50 and 25 mg/kg. PD samples were collected at multiple points during the study (4 and 24 hr post dose 1, 4 hr post dose 3, 8, & 10). The high dose had to be discontinued after 8 administrations due to body weight loss.



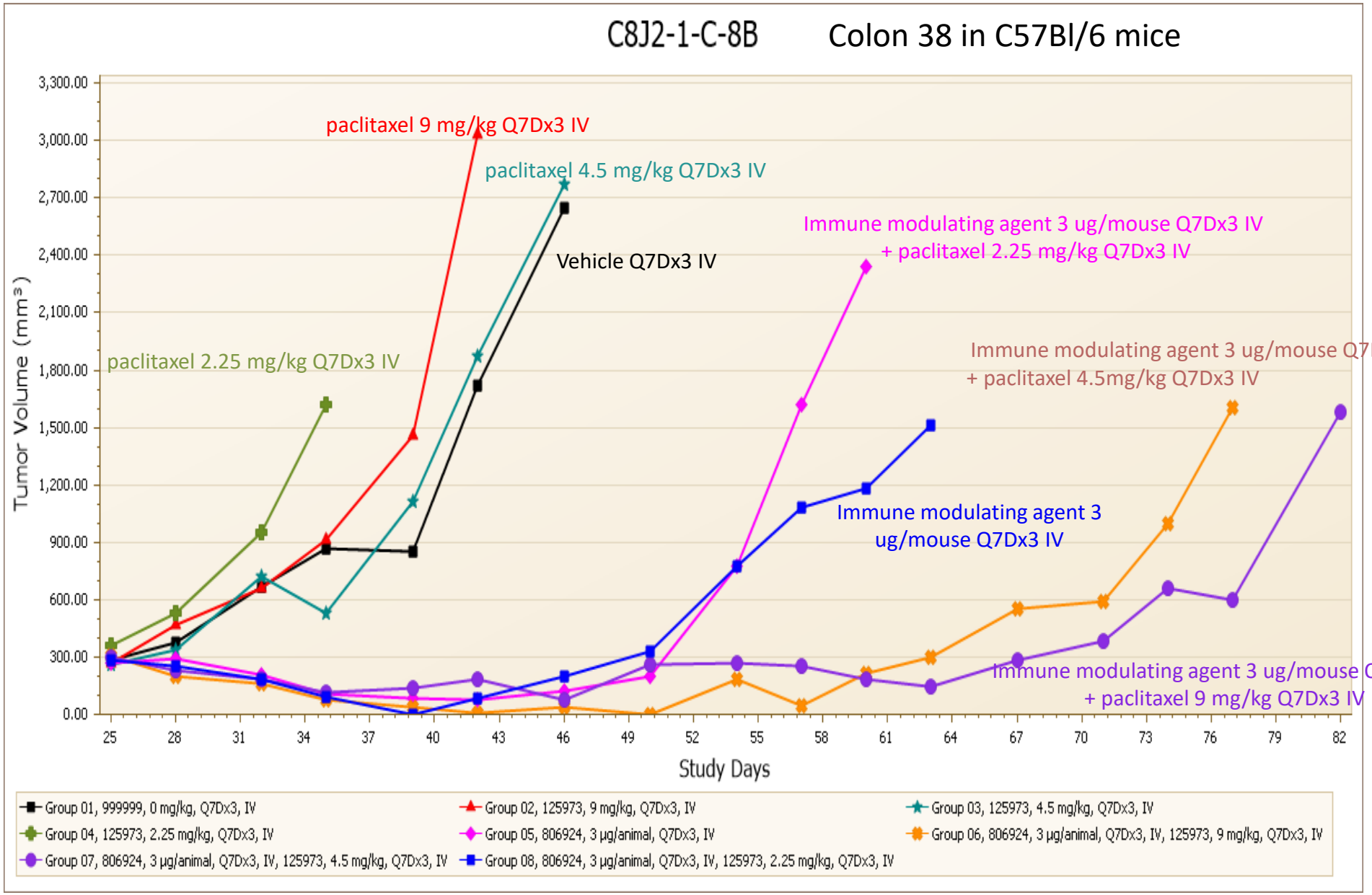


https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7802886/

What about immunologically active agents?

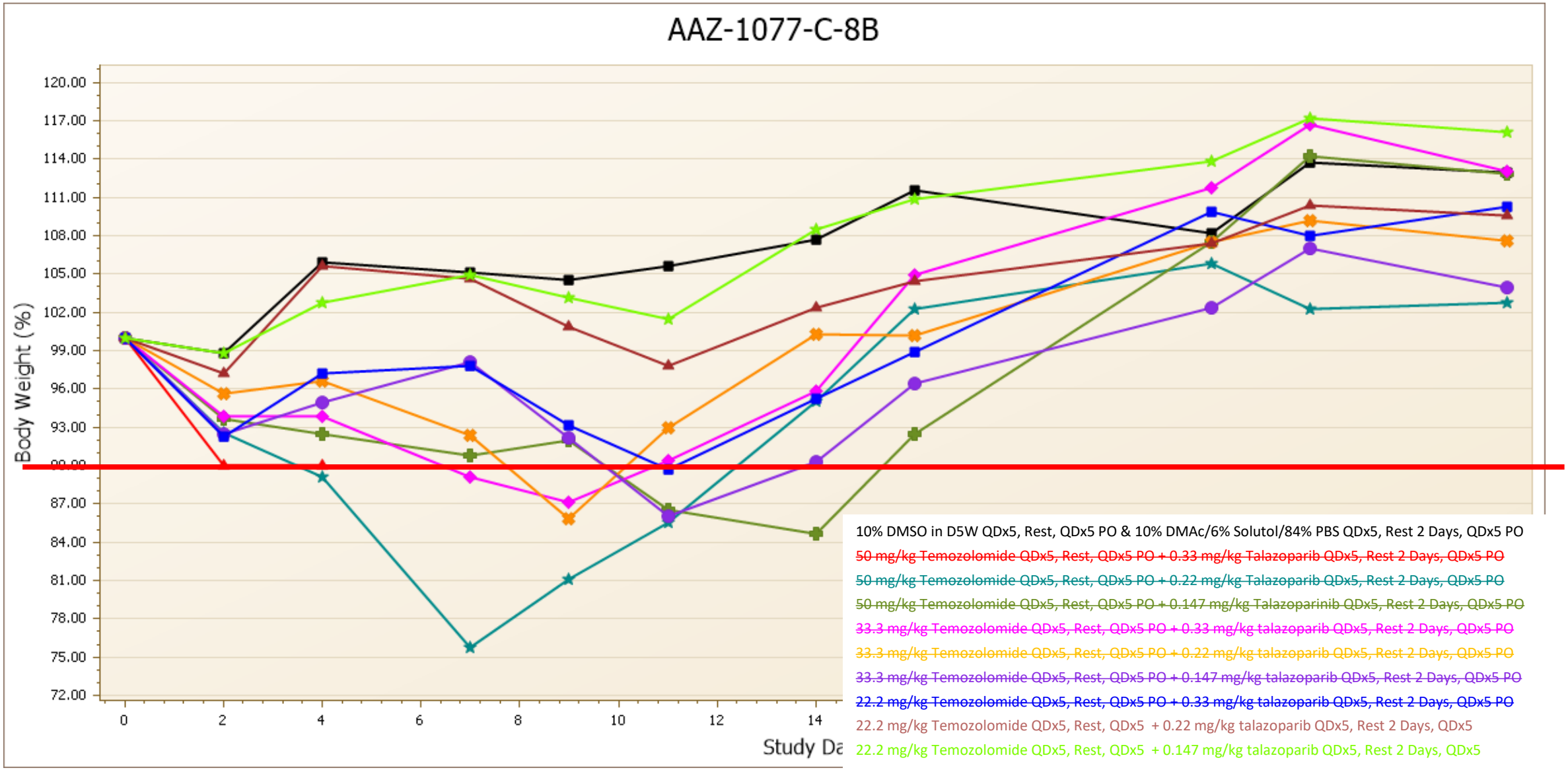
An immune competent model and drug dosing schedules different from those for many small molecules are likely needed

- Half-life
- Mechanism
- Human vs mouse target similarity
- Host response to therapeutic

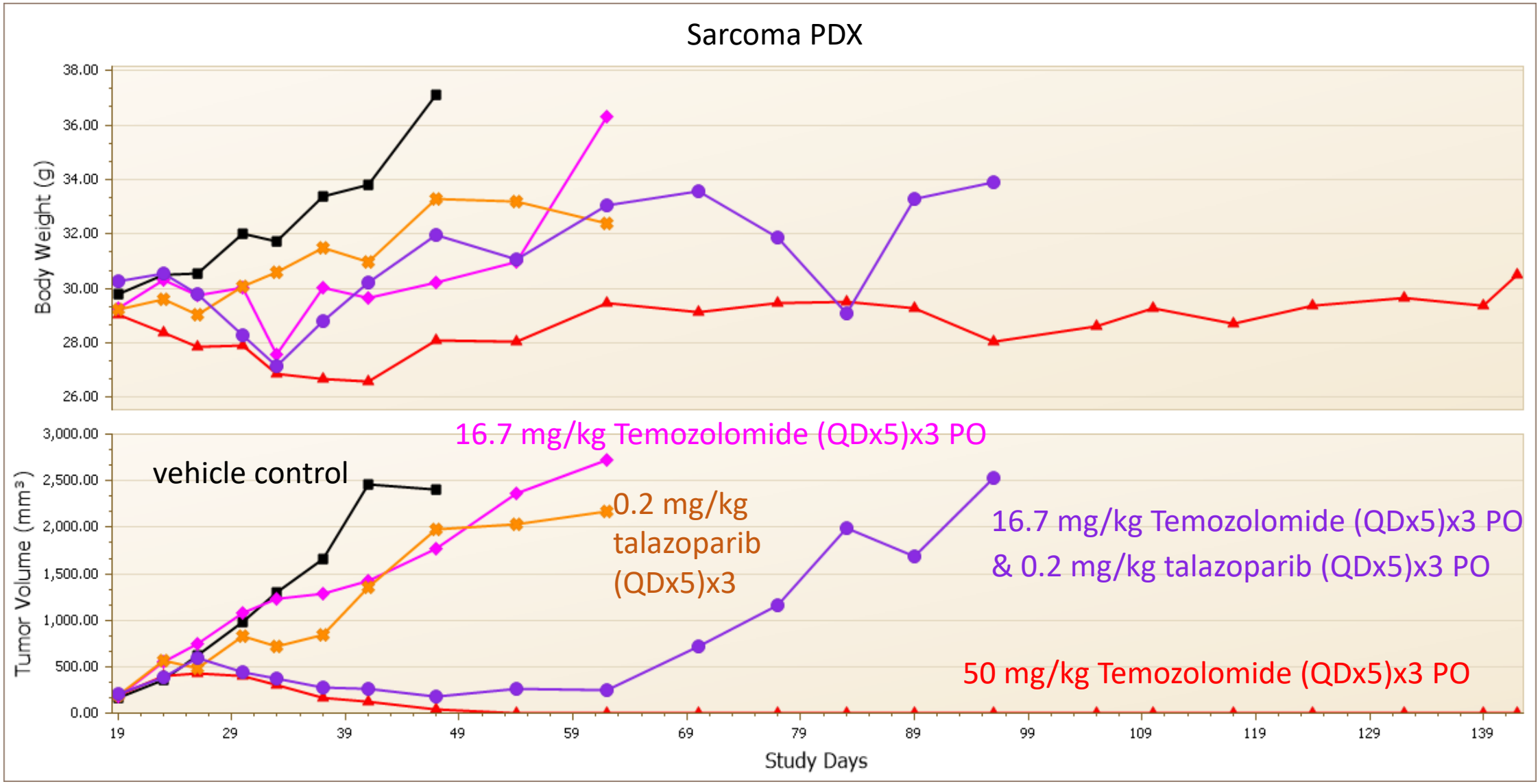


What about testing a combination of 2 drugs

First step is tolerability determination



Assess the effect of the 2 agents alone and in combination in relevant models



Variability inherent in the methods used to study tumors

Operator injection and/or surgical skill

Caliper measurement variability

Positioning for bioluminescence data capture

Implantation site/method

Clumping of cells being injected particularly for IV & orthotopic implants

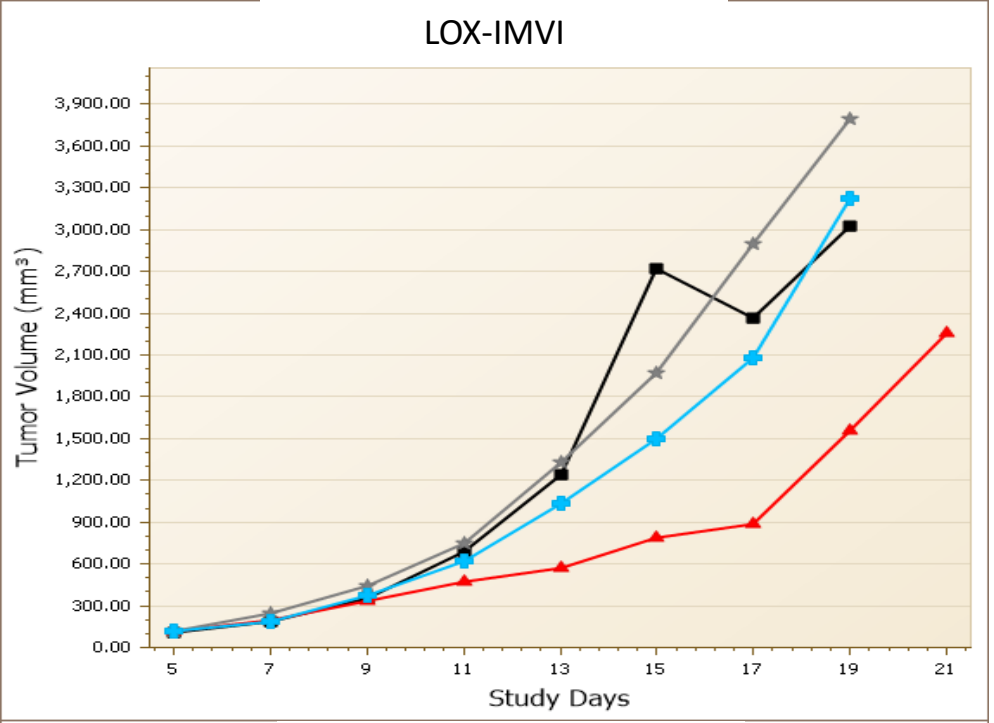
Drug prep errors

Using average mouse group weights rather than individual body weights for dosing determinations

Technical aspects of the animal work require consideration and establishing standards

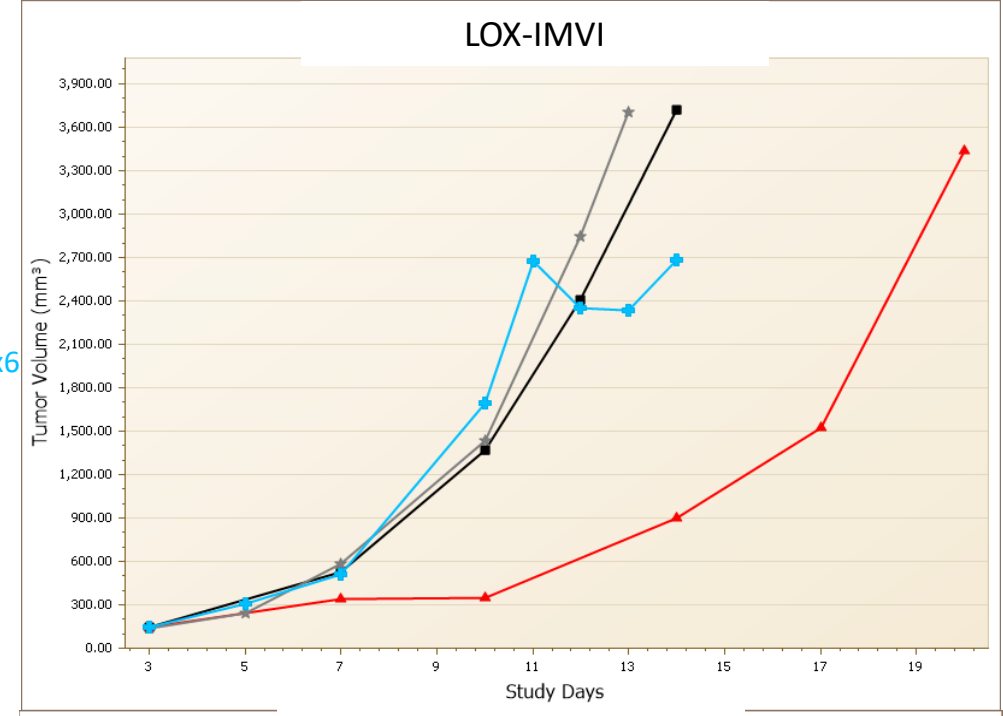
Note the difference in lung metastasis counts between the 2 operators with the passage 10 cells and between the passage 2 and passage 10 cells. Identifying and controlling as many variables as possible improves study outcome and reproducibility

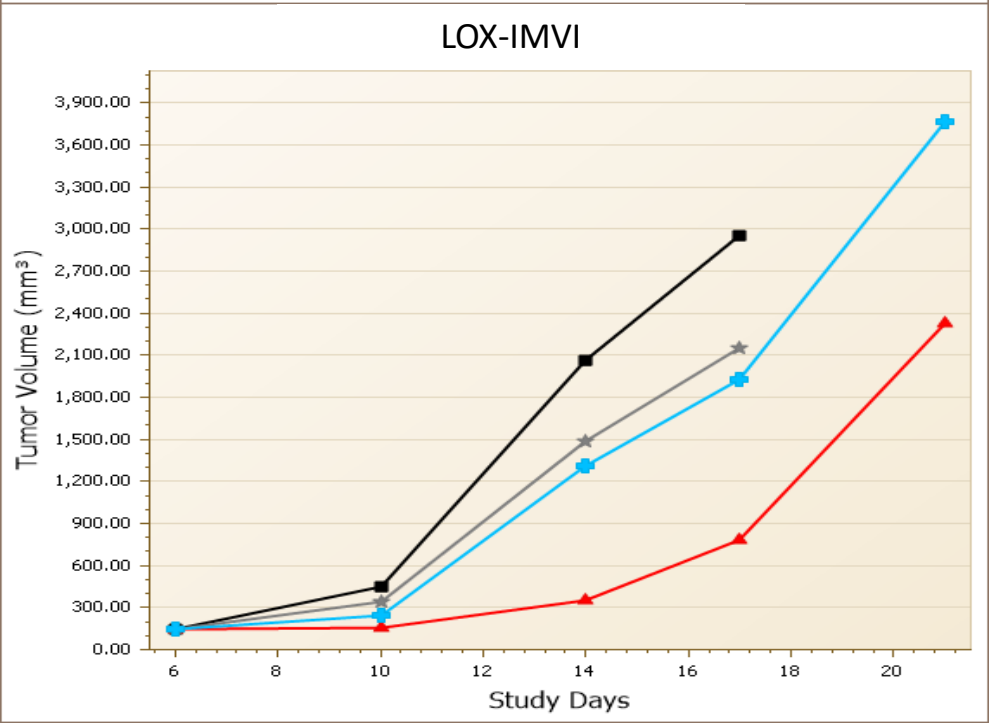
	C57Bl/6 mice			C57Bl/6 mice	
	1x10e5 passage 10			1x10e5 passage 2	
	tech 1	tech 2		tech 1	tech 2
	137	111		170	106
	245	11		316	257
	72	48		184	231
	87	75		196	323
	146	55		256	350
	78	111		183	114
	73	56		138	287
	80	36		142	236
	165	79		261	229
	116	60		134	306
	110	43		155	312
	173	111		274	314
	75	55		217	86
	102	88		159	100
	213	27		192	188
	62	94		259	189
	162	82		458	130
	154	37		259	402
	100	53		291	282
	128	47		233	167
average	124	64		224	230



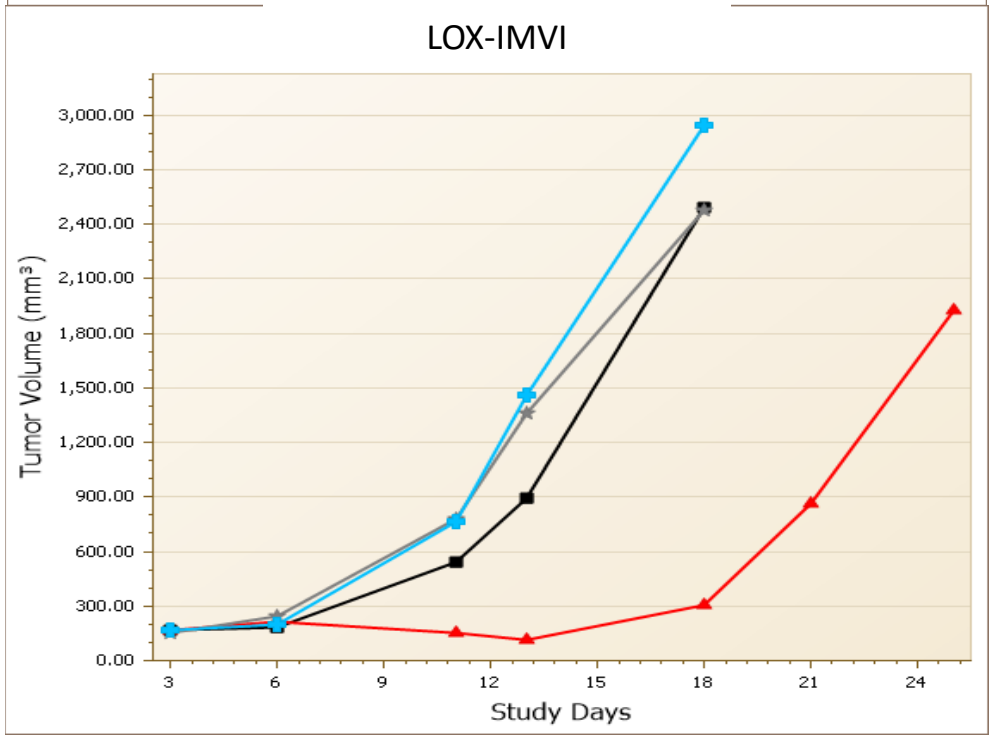
Treatment groups:

0.9% saline Q4Dx3 IP
5 mg cisplatin/kg Q4Dx3 IP
10% ethanol in saline Q2Dx6 SC
TNP-470 in 10%ethanol/90% saline Q2Dx6





4 experimental replicates conducted with the same test compounds against the same tumor model implanted from the same cryopreserved cell stocks with 4 different operators



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. Strength is added to any drug development effort when multiple, distinct preclinical models are demonstrably sensitive to the test article. If the range of antitumor activity is narrow take time to understand what the agent is doing so you can justify moving forward toward clinical trials through the identification of an appropriate patient population.