Animal Models of Disease States
Chapter 7- Blass

Prof M Kaur

Stages of Drug Discovery

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	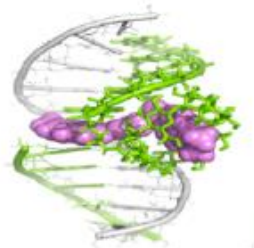				
Disease identification	Therapeutic Target	Lead Identification -High throughput Screening -*In vitro* and *in vivo* pharmacology ...	Preclinical trials -Animal test -HTS	Clinical Trials	FDA Approval

High-throughput screening (HTS):
Lead identification and Preclinical toxicology

http://www.ibd.utm.my/wellness2012/pdf/presentation/ICBWI2010_D1P03_Prof_Dr_Shang_Tian_Yang.pd

Primary Bioassays

- Cell-based Bioassays
- Many other *In Vitro* bioassays/assays

Secondary Bioassays

- **Animal-based assays (*In Vivo*)**
- Toxicological Assessments in whole animals
- ADME Studies
- Behavioral Studies
- Preclinical Studies

A hit rate of 1% or less is generally considered reasonable

https://www.iccs.edu/images/uploads/Dr-I-Bioassay%20Course.ppt

In Vitro Bioassays

In Vitro: In experimental situation outside the organisms. Biological or chemical work done in the test tube (in vitro is Latin for "in glass") rather than in living systems

- *Toxicity Assays*
  - MTT assay
  - Cancer cell line assays

In Vivo Screenings

In Vivo: Test performed in a living system such as antidiabetic assays, CNS assays, antihypertensive assays, etc.

- *Animal Toxicity*
  - Acute toxicity
  - Chronic toxicity
- Pre-Clinical Trials
- Clinical Trials

https://www.iccs.edu/images/uploads/Dr-I-Bioassay%20Course.ppt

Creating a virtual tumour cell

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Virtual Reality Tumour

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CREATING A VIRTUAL REALITY TUMOUR

IMAXT: imaging and molecular annotation of xenografts and tumors

https://www.ast.cam.ac.uk/content/virtual.reality.tumour

Physicochemical properties of compounds, such as their propensity to undergo metabolism, inhibit the metabolism of other compounds, or move across biological barrier can be predicted using *in vitro* models. Given the wide range of biological and physical properties that can be measured in an *in vitro* setting, **why is it necessary to study potential drug compounds in animal models?**

FDA and other regulatory agencies require both proof of efficacy and safety in animal models before they will allow a compound to be studied in humans.

In vitro is only an incomplete model of a far more complicated system. In whole animals, the net biological impact of a compound is the sum total of the compound's effect on all of the macromolecules, tissues, and organs that it comes into contact with.

Pharmacokinetic (PK) and Pharmacodynamics (PD)

- the pharmacokinetic profile of a compound provides a wealth of information on what happens to a compound when it enters the body.
- Assessing the biological impact of a compound in an intact animal requires more complex models that are designed to determine the PD of a compound. In simple terms, pharmacodynamics is the study of what a compound does to the body
  - EFFICACY- capacity of a compound to produce a desired biological endpoint
  - POTENCY- the amount of compound required to produce an effect of a given intensity
- The most commonly reported value for an *in vivo* study is its ED_{50}, the dose at which 50% of the maximum effect is observed.

Therapeutic Index (TI)

- Drug will have desired and non-desired effects (side-effects)
- Knowledge of the dose dependency of both the desired and undesired biological responses allows the determination of therapeutic window in which the positive effect is elicited while the negative effect is minimized. The ratio of these two doses is referred to as the therapeutic index.
- Thus, if a compound lowers cholesterol effectively at 1 mg/kg, but causes heart rate to drop at 10 mg/kg, then the therapeutic index is 10.
- If the same compound causes hair loss at 100 mg/kg, then the therapeutic index for this effect is 100,

A higher therapeutic index is generally considered the most important in a drug discovery setting.

SOURCES OF ANIMAL MODELS

- Insertion of non-native DNA or the suppression of normal gene function (knockout) via transgenic technology

e.g. The study of amyotrophic lateral sclerosis (ALS), also known as Lou Gehrig's disease, for example, has been significantly enhanced by the development of the SOD1-G93A transgenic mouse model.

- Drug-induced animal models

e.g. Administration of 1-methyl- 4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) leads to rapid destruction of dopamine-synthesizing neurons in the substantia nigra region of the brain. This triggers the rapid development of Parkinson's disease symptoms in primates and mice.

SOURCES OF ANIMAL MODELS

- produced by physical means (e.g., mechanical, surgical, etc.).

e.g. Ischemic events can be surgically created by limiting or blocking blood flow in order to study the impact of test compounds on stroke survival or cardiac reperfusion after a heart attack.

An animal model that does not correlate with a human disease or condition is of little value for the development of novel therapies.

CATEGORIES OF ANIMAL MODELS

homologous, isomorphic, and predictive

- Homologous animal models are

- the most desirable, as they have the same causes, symptoms, and treatment options available for humans.
- are the rarest type, as they are difficult to achieve.
- Typical example include the non-obese diabetic mouse model of type 1 diabetes
- The majority of animal models are not homologous models

- Isomorphic animal models are

- has the same symptomology as the human conditions and treatment options are generally the same. However, the root cause of the disease or condition in the animal model is not the same as that observed in humans are far more common.
- Typical examples include the animal models of stroke

- Predictive animal models are

- In some cases, the animal model itself may have little or no obvious resemblance to the human condition, but there are facets of the model that allow researchers to use it as a predictive tool.
- Typical example include schizophrenia.

SPECIES SELECTION

- Mice, Rat or Primates
- Non-human primates are, of course, the closest animal to humans overall, but they are rarely used in animal trials.

Limitations-

- Very few non-human primates available for study,
- are both difficult and expensive to maintain.
- Their large size impacts compound supply issues, as potential therapeutics are most often dosed on a milligram per kilogram basis.
- Larger animals require larger amounts of compounds, further driving up the expense of non-human primate studies.
- Additional ethical considerations also come into play.
- Non-human primates are typically used only when no other option is available.

Numbers to be included- an important aspect

International Agency for Research on Cancer (IARC), there were 14.1 million new cancer cases and 8.2 million cancer-related deaths in 2012 worldwide. These figures are expected to grow to 21.4 million new cases and 13.2 million cancer-related deaths by 2030 as the population grows and ages.

https://www.cansa.org.za/cancer-statistics/

ANIMAL MODELS OF ONCOLOGY

The National Cancer Institute's cancer model database contains over 6000 models as of 2013

Selecting the proper animal model is critical to the success of an oncology program

Mouse Xenograft Tumour Model

athymic nude mice or severely compromised immunodeficient (SCID) mice

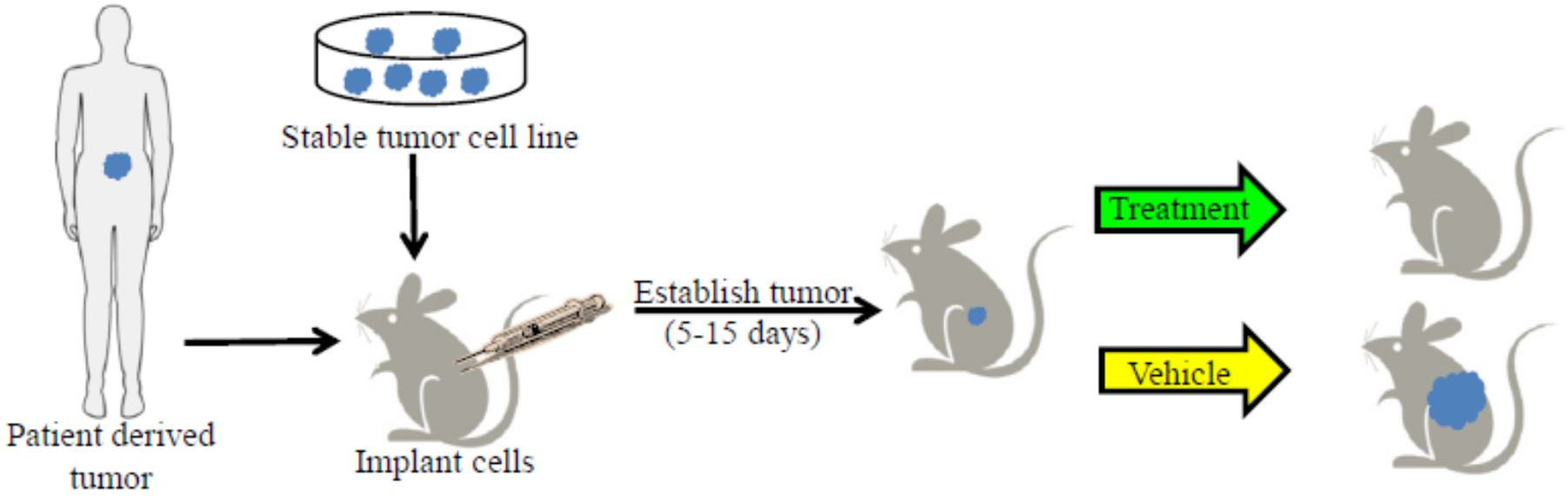
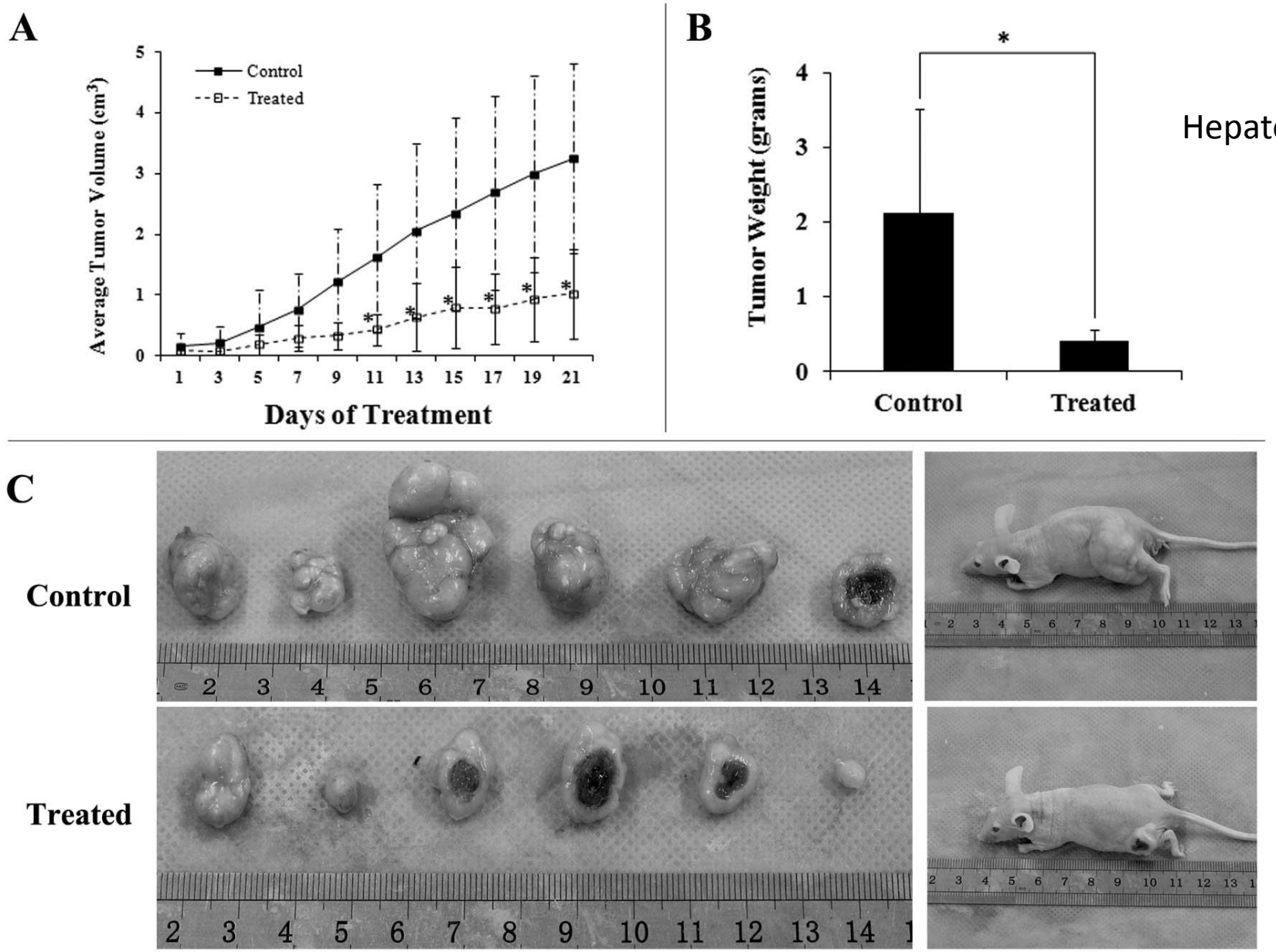


FIGURE 7.18 Mouse xenograft models can be used to determine the antitumor efficacy of candidate compounds. A tumor is established in an immunocompromised mouse using either patient-derived tumor cells or a stable tumor cell line. Once the tumor is established, treatment with a candidate compound or vehicle is initiated. Changes in the tumor size are monitored to determine the efficacy of candidate compounds.

Xenograft models using standardized cell lines has been a workhorse of cancer drug discovery programs for several decades

Athymic- means lack thymus gland so have no T cells



Hepatocellular carcinoma

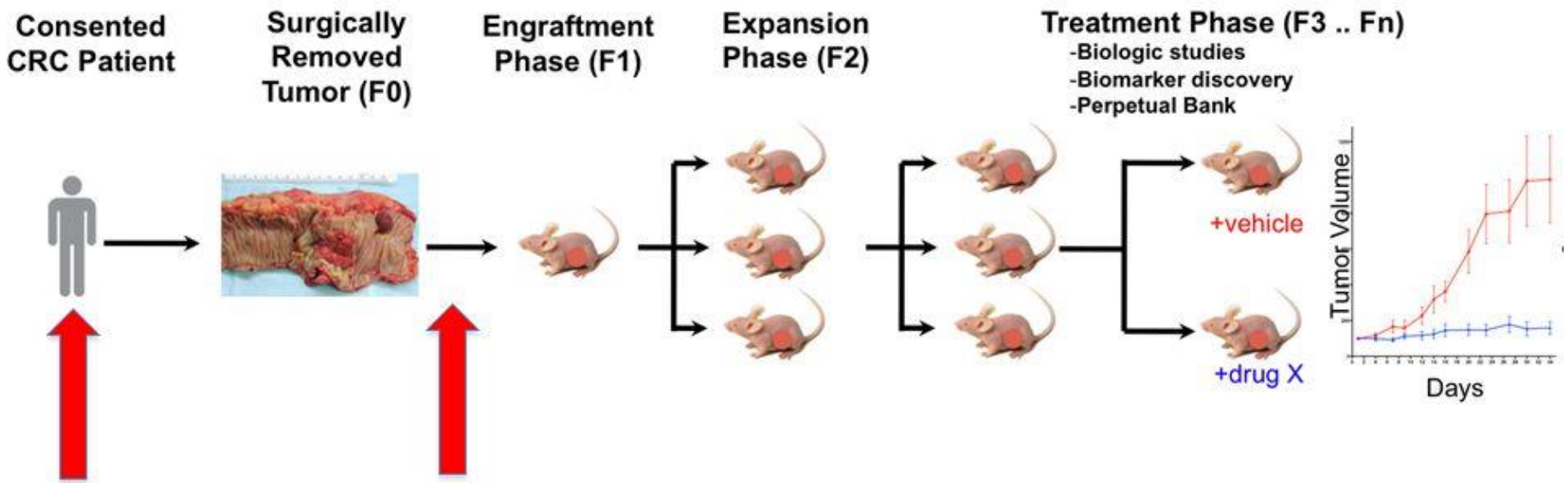
Courtesy: https://www.google.co.za/imgres?imgurl=https%3A%2F%2Fwww.spandidos-publications.com%2Farticle_images%2Fijo%2F40%2F4%2FIJO-40-04-1298-g03.jpg&imgrefurl=https%3A%2F%2Fwww.spandidos-publications.com%2Fijo%2F40%2F4%2F1298&docid=IuLSyNPfm125qM&tbnid=Zlxww25m0o6rdM%3A&vet=12ahUKEwjNvq_RxNPdAhVID8AKHVgbDPU4ZBAzKAIwAnoECAEQAw..i&w=1913&h=1715&bih=657&biw=1396&q=assignment%20xenograft%20mouse%20model%20undergrad&ved=2ahUKEwjNvq_RxNPdAhVID8AKHVgbDPU4ZBAzKAIwAnoECAEQAw&iact=mrc&uact=8#h=1715&imgdii=roepCwcn18jWYM:&vet=12ahUKEwjNvq_RxNPdAhVID8AKHVgbDPU4ZBAzKAIwAnoECAEQAw..i&w=1913

Question: Can we use patient tumour sample to induce tumour in xenograft mouse model?

- 1. Yes
- 2. No

Nude mouse

Schema: Patient Derived Xenograft Model (PDX)



These are the logistical steps that need to be worked out: consenting patients and getting tumor from pathology in OR

Note: Tumors are never grown on plastic

Courtesy:
https://www.google.co.za/imgres?imgurl=https%3A%2F%2Fimages.slideplayer.com%2F25%2F7817473%2Fslides%2Fslide_2.jpg&imgrefurl=https%3A%2F%2Fslideplayer.com%2Fslide%2F7817473%2F&docid=8uI3-6_daHkDvM&tbnid=sKX3uRIwMClkBM%3A&vet=10ahUKEwiRtMez0NPdAhUH_qQKHXi6DHkQMwiTAShNME0..i&w=960&h=720&bih=657&biw=1396&q=xenograft%20model%20students%20lab&ved=0ahUKEwiRtMez0NPdAhUH_qQKHXi6DHkQMwiTAShNME0&iact=mrc&uact=8

Limitations of xenograft model

- is the use of cross-species transplantation (human tumour cells to a mouse host).
- The experiment requires the use of immunocompromised mice in order to ensure that the human tumour cells will not be rejected by an immune response mounted by the host animal.

- Problem: above do not follow natural progression of cancer in humans.
- Solution: The allograft mouse model provides an opportunity to study potential antitumor agents in the presence of a normal immune system.

Mouse Allograft Tumour Model

Immunocompetent mice (those with an intact immune system) are subjected to experimental conditions

- but the human tumour cells are replaced with mouse tumour cell lines (an intraspecies transplantation rather than an interspecies transplantation).
- Candidate compounds can be introduced in the same manner as described for the mouse xenograft model, and their efficacy can also be determined in the same manner (changes in tumour size over time).
- Problem: use of mouse cancer cells and not human cells
- As a result of this issue, allograft models are less utilized than the xenograft models in cancer drug discovery programs

QUESTION

- Do xenograft and allograft models represent the microenvironment of naturally-occurring tumours?

1. Yes
2. No

Genetically Engineered Mouse (GEM) Models of Cancer

- Answer is ‘NO’. Factors that would influence natural tumour cell growth and tumour formation are simply not present in these models.
- In GEM models, genes that are suspected of participating in the transformation of normal cell into malignant cells and tumours are targeted for mutation, overexpression, or deletion.

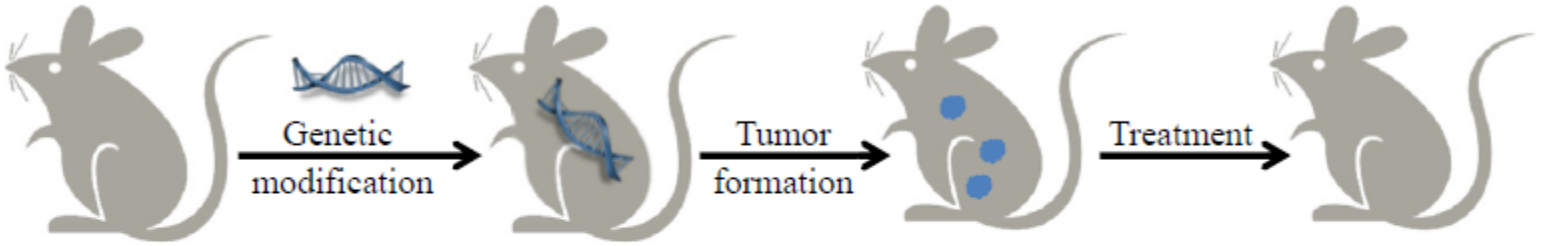


FIGURE 7.19 Genetically engineered mice are created using transgenic techniques. In one method, genes that promote spontaneous tumor formation are inserted into otherwise normal mice. Candidate compounds with *in vivo* antitumor properties will cause the tumors to shrink and/or decreased in number.

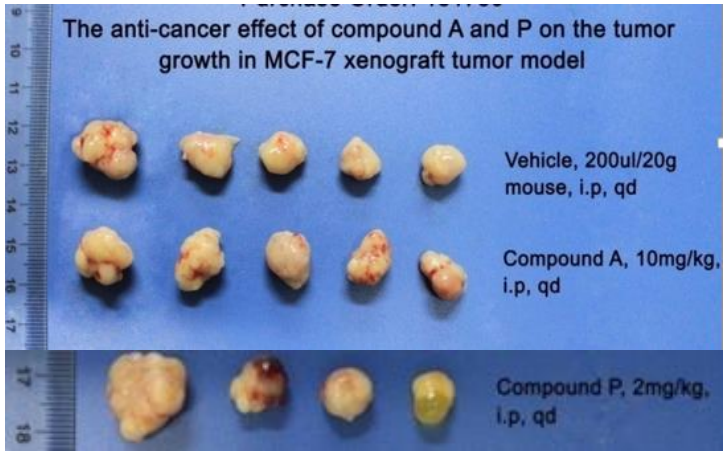
Benefits of using GEM model

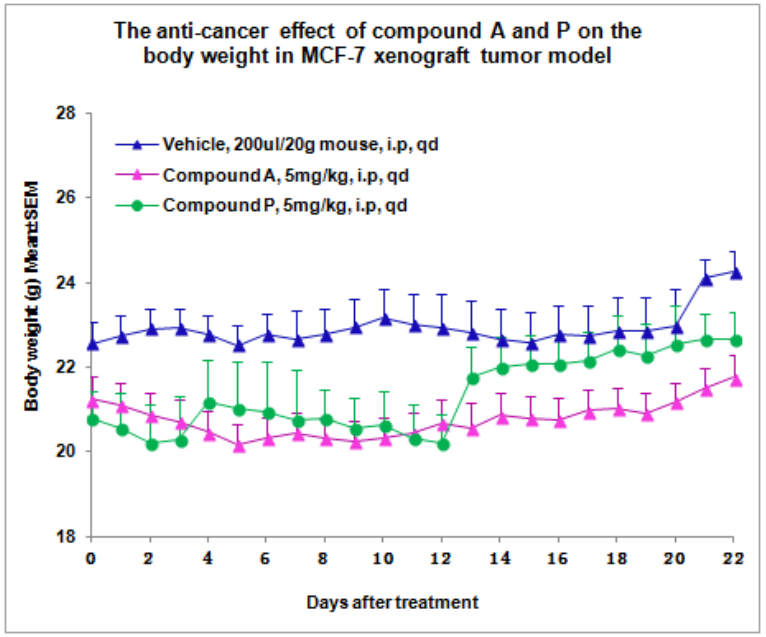
- Various stages of tumour progression can be studied.
- determine the impact of therapeutic agents at each of these stages.
- tumour microenvironment of GEM models more closely mimics natural cancer progression.

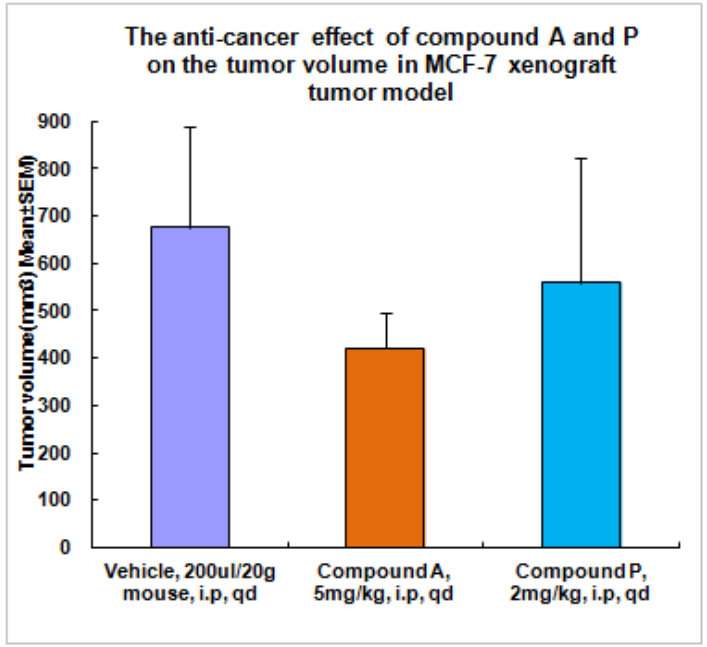
DISADVANTAGES

- costly and time-consuming.
- GEM tumours are typically the result of limited alteration of the mouse genome, human tumours are heterogeneous in nature (Multiple mutations may be present in a clinical setting).
- tumours that develop in GEM models are mouse tumours, not human tumours.
- As a result, efficacy in a GEM model is not necessarily predictive of what will happen in a clinical setting.

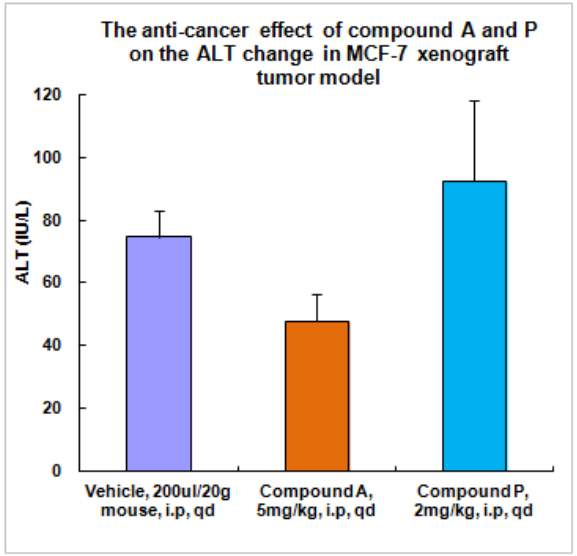
Interpretations

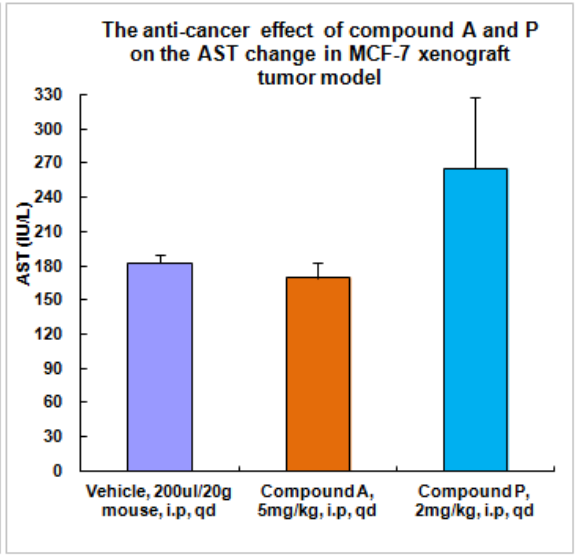






Liver Toxicity markers- Activity of ALT (alanine aminotransferase) and AST (aspartate aminotransferase).





Interpretations-Kaplan-Meier Survival curve

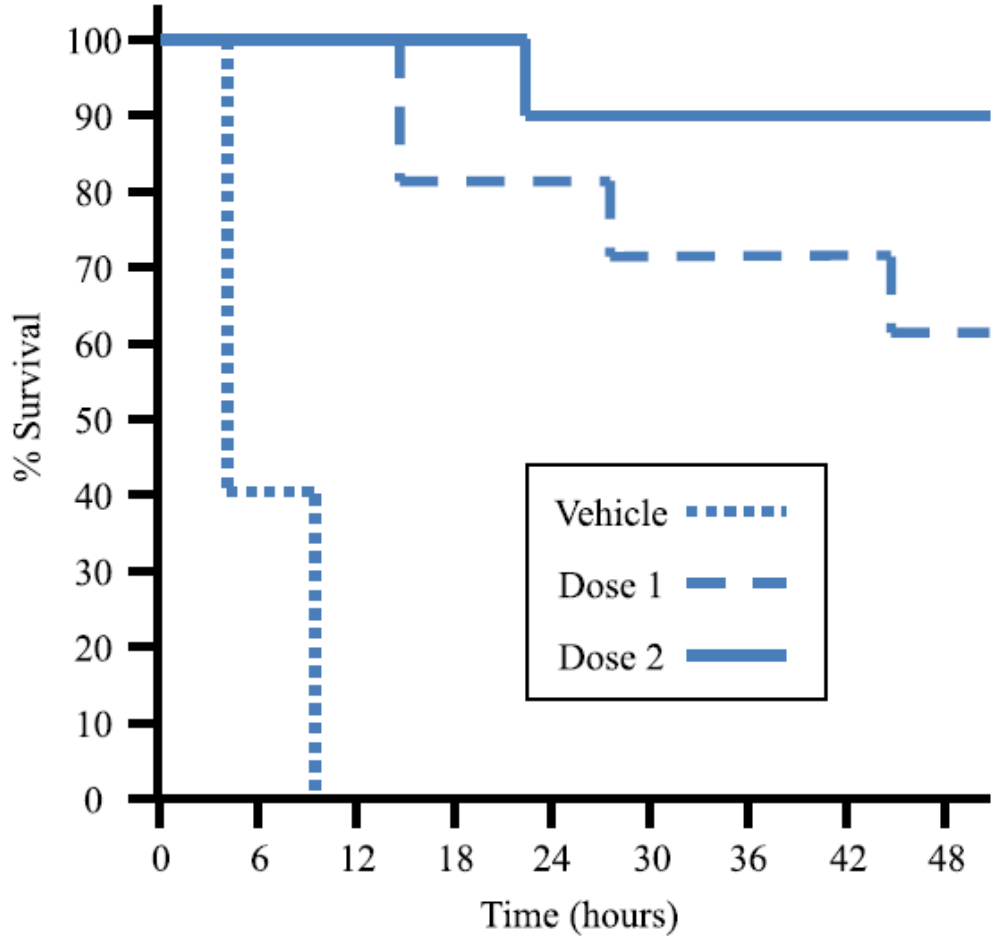


FIGURE 7.17 A Kaplan–Meier survival curves can be used to determine the efficacy of various doses of a candidate compounds in an infection model. In this graph, infected mice treated with the vehicle die faster than mice treated with a candidate compound at two different doses.

can be used to compare the efficacy of multiple compounds or dose levels

Extra resources

- https://www.animalethics.org.au/education-and-training/educational-material