# What are animal model challenges for the development and evaluation of cancer nanotherapeutics?

## It's Not the Animal Model, Inadequate.
## It's the Human Use, Inadequate

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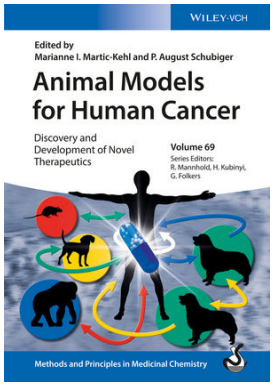
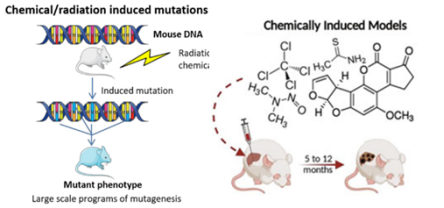
# Animal Models for Cancer Studies

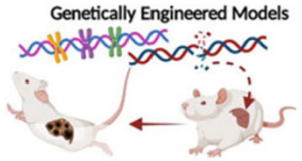


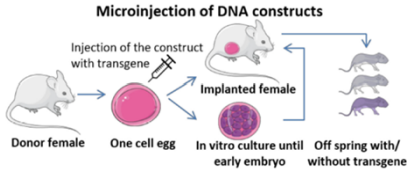


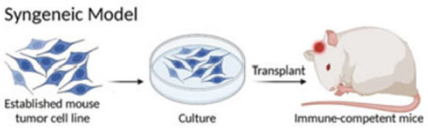


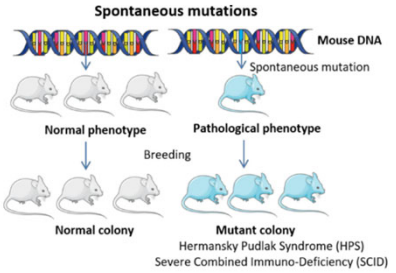


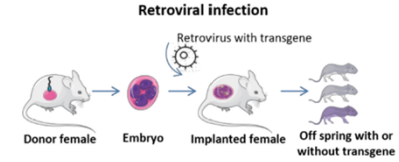


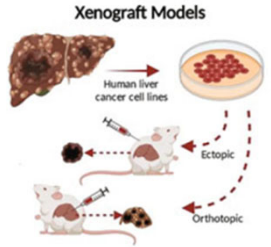


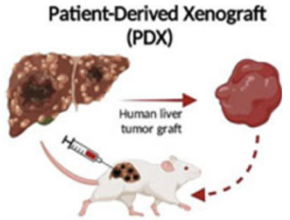




Commonly used animal models for cancer studies.
(Dhumal et al., Preclinical animal models for cancer research and drug discovery, in Bose & Chaudhari, Eds., Unravelling Cancer Signaling Pathways. 2019.)
https://www.eara.eu/why-are-animals-used-cancer-research

Das et al., Importance of animal models in the field of cancer research & Karakurt et al., Animal model of human cancer: malignant lymphoma/ colon cancer/lung cancer/liver cancer/brain tumors/skin cancer (in Pathak 2023, Handbook of Animal Models and its Uses in Cancer Research)

# A Common Perception in the Nanomedicine Field

#1

**Perception:** **Current animal models are inadequate for the development of nanomedicine.**

**Facts:** Animal models are fine for their intended uses.

**We have unreasonably high expectations for nanotherapeutics**.

**Nanomedicine is a vague, subjective term.**

| Term | Introduced in | FDA Approval | Formulation | Nanomedicine? |
|---|---|---|---|---|
| • Liposomes | 1964 | Doxil (1995) | PEGylated liposome | No→Yes |
| • Polymer Micelles | 1965 | Taxol (1992) | Polymer micelle (Cremophor EL) | No |
| | | Taxotere (1996) | Polymer micelle (Polysorbate 80) | No |
| • Nanoparticles | 1976 | Abraxane (2005) | Albumin-drug conjugate | Yes |
| | | Onpattro (2018) | PEGylated Lipid Nanoparticle | Yes |
| • PEGylation | 1977 | Adagen (1990) | PEGylated protein | Yes |
| • Antibody-Drug | 1983 | Mylotarg (2000) | Antibody-drug conjugates | Yes |
| • Nanocrystals | 1995 | Rapamune (2000) | Drug crystals in nanosize | Yes |

# Another Common Perception in the Nanomedicine Field

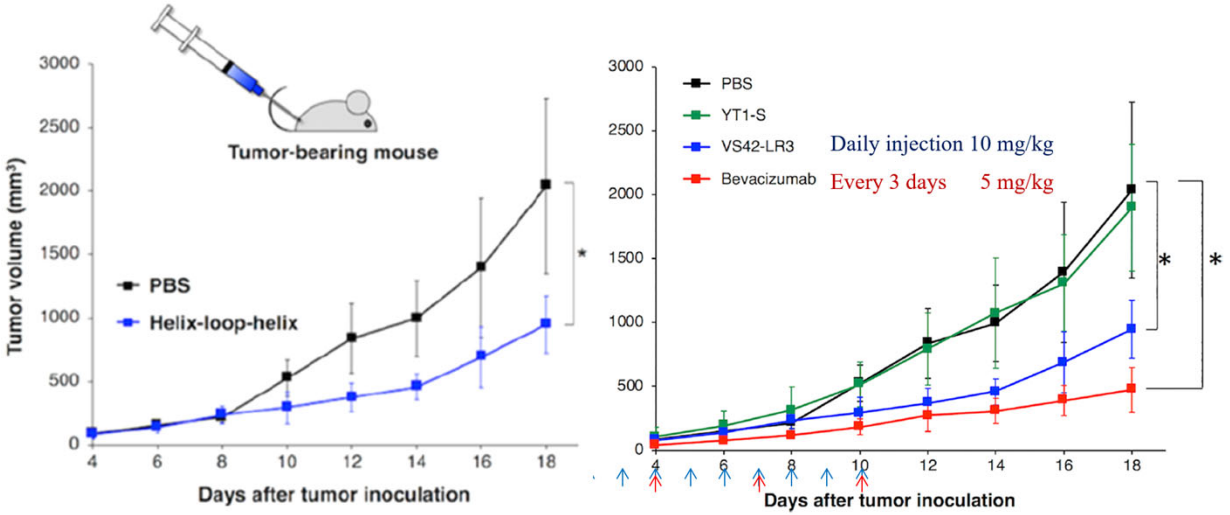
#2

**Perception:** **Nanomedicines work in animal models but not in humans.**

**Facts:** **Nanomedicines do not work in animal models either.**

Publications on Nanomedicines (Nanotherapeutics)

- Controls are almost always buffers (e.g., PBS): "Better than buffer" has little meaning.
- The new formulations should be superior to **standard therapy** with no side effects!
- "Equivalent" or "non-inferior" to an available standard therapy is not an improvement.



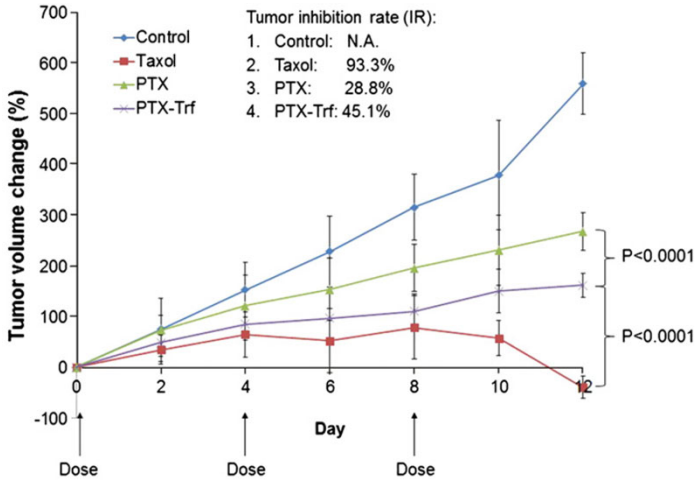




**Fig. 7.** *In vivo* antitumor efficacy of PTX formulations in mice.

Drug needs to be absorbed into the tumor cells, rather than the amount deposited near the tumor

# The EPR Effect: The Source of All Confusion in Nanomedicine

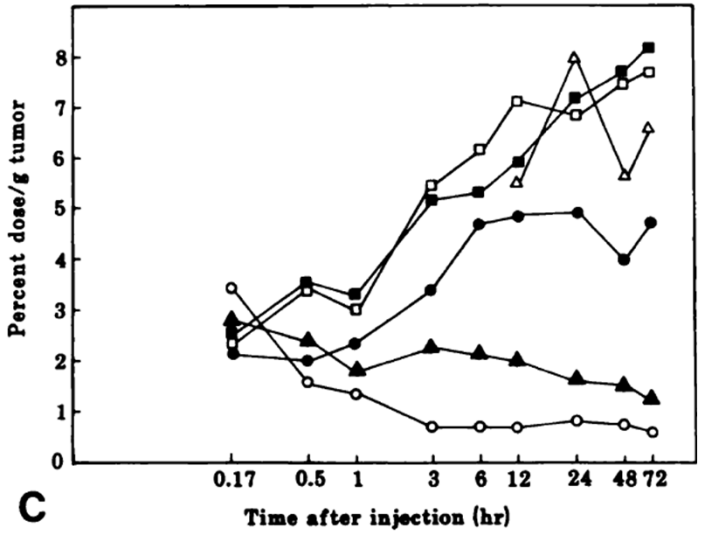
**SMANCS**: conjugates of styrene and maleic acid copolymer (SMA) and neocarzinostatin (NCS).
The molecular weight of NCS is 12,000 Da and two SMA chains of 2,000 Da each were conjugated to make the 16,000 Da molecule.

Table 3 *Tissue distribution of various $^{51}$Cr-labeled proteins in tumor-bearing mice after i.v. injection*

| | Proteins recovered as % of injected doses/g of specimen at 3 different times (h) | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Neocarzinostatin | | | Smancs | | | Ovomucoid | | | Bovine albumin | | | Mouse albumin | | | Mouse IgG | | |
| Tissue/organ | 24 h | 48 h | 72 h | 24 h | 48 h | 72 h | 24 h | 48 h | 72 h | 24 h | 48 h | 72 h | 24 h | 48 h | 72 h | 24 h | 48 h | 72 h |
| Tumor | 0.82 | 0.75 | 0.6 | 4.92 | 4.0 | 4.71 | 1.66 | 1.51 | 1.26 | 6.85 | 6.95 | 7.22 | 7.15 | 7.68 | 8.18 | 7.98 | 5.65 | 6.56 |
| Blood | 0.35 | 0.24 | 0.28 | 0.86 | 0.49 | 0.24 | 0.27 | 0.18 | 0.13 | 4.06 | 1.90 | 0.95 | 5.18 | 2.84 | 1.52 | 5.27 | 2.28 | 2.24 |
| Liver | 10.46 | 12.25 | 15.12 | 42.42 | 50.71 | 42.92 | 15.67 | 11.3 | 12.58 | 16.05 | 13.28 | 15.09 | 17.56 | 37 | 18.12 | 6.88 | 2.56 | 4.55 |
| Kidney | 4.78 | 4.48 | 3.79 | 2.43 | 2.8 | 2.75 | 7.77 | 6.6 | 4.24 | 4.17 | 3.46 | 3.47 | 6.21 | 4.37 | 3.81 | 5.15 | 2.25 | 2.00 |
| Spleen | 6.29 | 8.9 | 9.57 | 24.21 | 40.40 | 36.4 | 3.26 | 3.17 | 2.85 | 6.09 | 5.63 | 4.7 | 6.51 | 5.97 | 5.1 | 4.09 | 1.91 | 2.88 |
| Lung | 0.68 | 0.91 | 0.41 | 2.36 | 2.98 | 2.13 | 0.78 | 0.55 | 0.49 | 2.47 | 1.76 | 1.6 | 3.48 | 2.34 | 2.42 | 4.36 | 1.91 | 2.12 |
| Heart | 0.42 | 0.51 | 0.36 | 1.25 | 1.58 | 1.70 | 0.77 | 0.84 | 0.74 | 1.80 | 1.53 | 1.17 | 2.34 | 2.31 | 1.77 | 2.4 | 1.25 | 1.58 |
| Stomach | 0.10 | 0.08 | 0.08 | 0.48 | 0.39 | 0.57 | 0.59 | 0.29 | 0.58 | 0.81 | 0.77 | 0.73 | 1.55 | 0.80 | 0.77 | 0.82 | 0.42 | 0.35 |
| Duodenum | 0.7 | 0.7 | 1.6 | 1.2 | 1.3 | 1.2 | 1.1 | 1.1 | 1.1 | 1.2 | 1.2 | 1.2 | 1.7 | 1.5 | 1.0 | 3.6 | 1.8 | 1.8 |
| Brain | 0 | 0 | 0 | 0.03 | 0.01 | 0.04 | 0 | 0 | 0 | 0.06 | 0 | 0.01 | 0.05 | 0.07 | 0.03 | 0.05 | 0 | 0 |
| Skin | 0.59 | 0.47 | 0.39 | 0.91 | 1.29 | 0.93 | 0.56 | 0.76 | 0.64 | 1.76 | 2.33 | 2.15 | 2.37 | 2.68 | 2.25 | 1.68 | 1.26 | 1.41 |
| Muscle | 1.1 | 1 | 0.9 | 0.9 | 1.7 | 1.2 | 0.7 | 0.8 | 0.8 | 1.0 | 1.3 | 1.1 | 1.2 | 1.4 | 1.5 | 2.7 | 1 | 2.8 |

We previously used smancs dissolved in lipid contrast medium and showed a marked retention of lipid (a T/B of 1200) in the tumor when we administered smancs/Lipiodol via a tumor-feeding artery (21). This, as a consequence, resulted in unprecedented clinical benefit with few side effects for patients with hepatoma and lung cancer (6, 7, 23). Furthermore, the method has diagnostic value: use of various X-ray systems permits a highly sensitive diagnosis, determination of subsequent dose regimen, and long-term follow-up (24). The basic mechanism operating here with lipid is again attributed to hypervasculature, enhanced permeability, little recovery from lymphatics, and perhaps an architectural uniqueness at the neovasculature level where more lipid adhered on the vascular endothelium than in normal counterpart.

All these data can be used to explain the general mechanism for the tumoritropism of smancs and γ-emitting metals used in radioscintigraphy for the diagnosis of solid tumors. Radioactive gallium or other γ-emitting metal citrates injected into the general circulation are bound to serum transferrin ($M_r$ 90,000) (25); the radioactive transferrin tends to accumulate more in the tumor. The highly tumoritropic properties of macromolecular anticancer agents as seen with smancs suggest a direction for the future development of new anticancer agents based on this prototype drug.




■ Mouse serum albumin
□ BSA
△ Mouse IgG
● SMANCS
▲ Ovomucoid
○ NCS

**Fig. 1. Plasma clearance and intratumor accumulation of various $^{51}$Cr-tagged proteins in tumor-bearing mice. Plasma clearance of various proteins with molecular weights ranging from 12,000 to 160,000 during short and long time periods is shown in *A* and *B*, respectively. Their intratumor concentration is shown in *C*. ○, NCS ($M_r$ 12,000); ●, smancs ($M_r$ 16,000); ▲, ovomucoid ($M_r$ 29,000); □, BSA ($M_r$ 69,000); ■, mouse serum albumin; △, mouse IgG ($M_r$ 160,000). Radioactive proteins were injected i.v. at time zero. Values are based on radioactivity. See text for detail.**


Y. Matsumura and H. Maeda, A new concept for macromolecular therapeutics in cancer chemotherapy: mechanism of tumoritropic accumulation of proteins and the antitumor agent SMANCS, Cancer Res. 46 (1986) 6387–6392.
Maeda et al.: Conjugation of poly (styrene-co-maleic acid) derivatives to the antitumor protein neocarzinostatin: Pronounced improvements in pharmacological properties, J. Med. Chem. 28: 455-461, 1985.

# The EPR Effect of the Tumor-Targeted Nanomedicine is an Illusion



**4 Challenges in Nanomedicine Clinical Translation**

Despite the uncountable attempts to develop targeted nanoparticulate therapies for drug delivery to tumors, few anticancer nanomedicines have been approved by regulatory agencies, thus generating a debate regarding the real effectiveness of these systems for cancer treatment. Most anticancer medicines follow the same two basic criteria when trying to design effective and safe sustained drug delivery systems based on lipid or polymeric nanoparticles: (1) the EPR effect, caused by the leaky vasculature next to the tumor, increases drug accumulation in the affected area, and (2) long systemic circulation of drug-loaded nanoparticles avoids the uptake by the RES, decreasing drug accumulation in the normal organs and reducing toxicity (Sun et al., 2020). The EPR effect influencing nanomedicines has repeatedly been confirmed, both in animal xenografts and in human cancer patients, using nanoparticle-encapsulated imaging agents (Gaillard et al., 2014; Greish, 2010; Hamaguchi et al., 2004; Koukourakis et al., 2000; Torchilin, 2011), but it is difficult to conclude if this EPR effect is different to the one observed for the free drugs. Free drugs, as small molecules with high plasma protein binding, also accumulate in tumors due to this phenomenon (Tang et al., 2014; Torchilin, 2011), and, due to ethical concerns, clinical trials with a free drug control arm are not possible in most cases; thus, there are very few direct comparisons between the free drug and the nanoparticle formulation.

When Doxil® reached the market, the accumulation of doxorubicin in patient tumors was found to be an order of magnitude higher than with free drug, and pathogenic analysis of KS revealed notably leaky vasculature (Northfelt et al., 1998; Uldrick & Whitby, 2011). However, in a later study, the evaluation of the tumor uptake of radiolabeled liposomes, with the same lipid composition as Doxil®, demonstrated considerable heterogeneity between patients with the same and different cancer types (Harrington et al., 2001). Since then, a few studies have demonstrated significantly higher drug concentrations in the tumors when administering liposomal formulations (Gabizon et al., 1994), but limited improvements have been the reason of failure and cancelation of many clinical trials (Dragovich et al., 2006; Kraut et al., 2005; White et al., 2006).

Recent studies increasingly downplay the EPR effect. An interesting analysis by Wilhelm et al., surveying the literature from the past 10 years, concluded that only 0.7% (median) of the administered nanoparticle dose is found to be delivered to a solid tumor (Wilhelm et al., 2016). Another meta-analysis found no significant difference in clinical anticancer efficacy between liposomal and conventional chemotherapeutics in terms of objective response rate, overall survival, and PFS (Petersen et al., 2016).

Another key aspect is the validity of the animal xenograft models to mimic the biological phenomena observed in human cancers. In the available animal models, the EPR effect is notably exaggerated, resulting in a poor clinical translation (Greish, 2010). Thus, there is an urgent necessity to develop new models for in vivo and in silico testing.

Regarding the long systemic circulation and the high plasma concentration, it can increase tumor accumulation if there is a strong EPR effect or decrease drug accumulation in normal organs to reduce toxicity. However, it can also reduce efficacy or alter drug distribution to different organs, generating new adverse events (Harrington et al., 2001; Ngan & Gupta, 2016; Northfelt et al., 1998).

In addition, even if nanoparticles are able to avoid clearance from blood circulation (by the mononuclear phagocytic systems or the RES, among others) and the shear stress caused by varying flow rates and extravasate next to the tumor, the complex extracellular matrix surrounding malignant cells will notably limit their penetration (Yuan et al., 1994). Furthermore, lack of drug release from the vehicles can significantly decrease drug availability (Laginha et al., 2005; White et al., 2006).

Furthermore, after hundreds of preclinical and a few clinical studies with actively targeted nanoparticles incorporating specific motifs directed to molecules that are usually overexpressed on cancer cells, none of the tested strategies have reached the market (Ernstoff et al., 2018; Mamot et al., 2012; Matsumura et al., 2004). This is probably linked to the fact that actively targeted nanosystems also rely on the same principles as the passive targeting until they reach the microenvironment of the tumor where they can match with the specific molecules on the cancer cell membranes, thus dealing with the same challenges.

In general, most of the marketed nanomedicines failed to show improved efficacy, in comparison with the reference treatment, but they significantly and consistently improved the toxicity profile of classic chemotherapeutic agents, allowing for the administration of higher doses and better patient quality of life (Batist et al., 2002; Drummond et al., 1999; Farokhzad & Langer, 2006).

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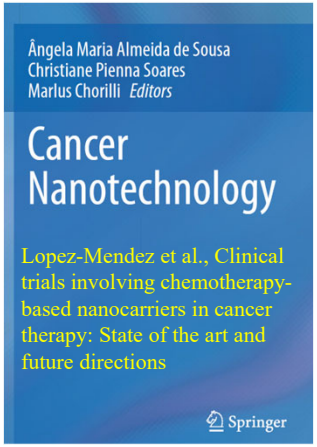


**"Nanomedicines" or "nanotherapeutics" are new names for old formulations.**

**The drug efficacy does not improve because of the name change.**

**We have unwarranted, unreasonably high expectations for nanotherapeutics.**

# Active Targeting by Nanomedicines does Not Exist

the efficiency of passive targeting. One of the important reasons for the poor application of passive targeting in humans is the large gap between animal models and humans. First, there are significant differences in the rate of tumor development, size ratio, metabolic rate, and host life cycle between mouse and human tumors, and there are also large differences in the microenvironment of tumors in different species.

In addition, the active targeting effect of the nano-preparations is questionable because only a small fraction of the intravenously administered active targeting nano-preparations accumulate at the tumor site, and thus their so-called "homing" mechanism is influenced by blood circulation. Secondly, the overexpression of receptors is not related to targeted delivery because normal cells also express these receptors, and the total number of normal cells is much larger than that of cancer cells, so most of the ligands are actually captured by normal cells, and the over-expression of receptors on cancer cells is actually not related to the increase in the number of nanoparticles to the tumor site [141].

nanoparticles do not rely on passing through the endothelial gap of tumor vessels (which can be up to 2000 nm in size) and that up to 97% of nanoparticles enter through the active process of endothelial cells tumors, the finding that actually raises questions about whether passive targeting can be achieved with nanoparticles and the efficiency of passive targeting. One of the important reasons for the poor application of passive targeting in humans is the large gap between animal models and humans. First, there are significant differences in the rate of tumor development, size ratio, metabolic rate, and host life cycle between mouse and human tumors, and there are also large differences in the microenvironment of tumors in different species.

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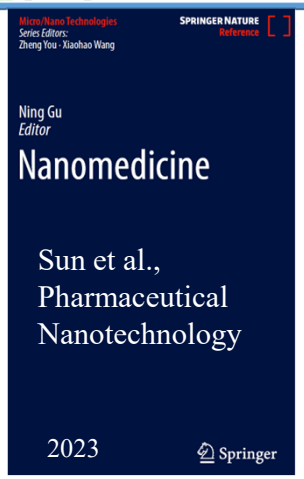
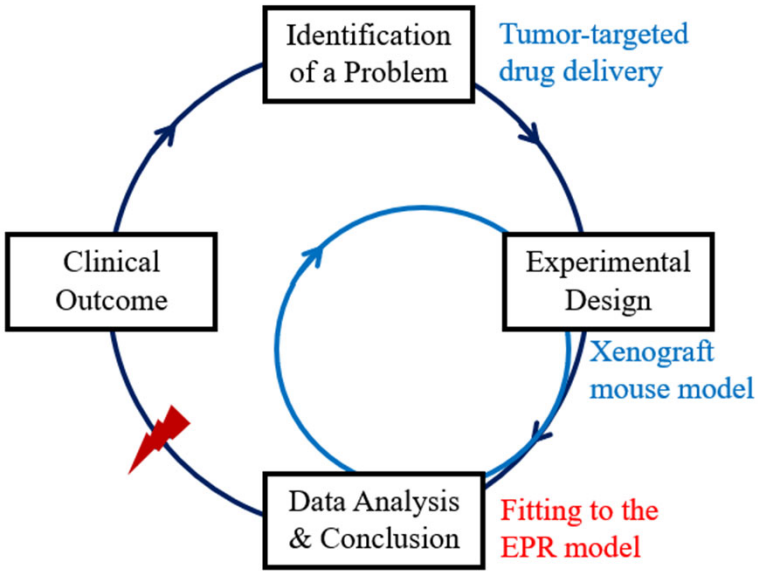
Nowadays, nanoparticles are mostly applied in basic research on antitumor and basic research on brain diseases treatment, and may be developed to more disease areas in the future, but the mechanisms for active targeting and passive targeting need to be further clarified, and in addition, in the process of engineering nano-particles, they may face the problems of difficult process control, poor reproducibil-ity, and strong amplification effect, which likewise make the clinical translation of nanoparticles appear to be difficult.




The xenograft mouse models are designed to obtain the data for the preconceived conclusion: Nanomedicine is better than the control.

# Animal Models Are Fine


Elaine A. Ostrander, Ph.D.
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Definition: An animal model is a non-human species used in biomedical research because it can mimic aspects of **a biological process or disease** found in humans. Animal models (e.g., mice, rats, zebrafish and others) are sufficiently like humans in their anatomy, physiology or response to a pathogen that researchers can extrapolate the results of animal model studies to better understand human physiology and disease. By using animal models, researchers can **perform experiments that would be impractical or ethically prohibited with humans**. --- Overall, animal models have proven valuable in studies of nearly every human condition.

(https://www.genome.gov/genetics-glossary/Animal-Model#)

# But Hypes are Dangerous

The lack of translation from animal studies to clinical studies is due to our unreasonable expectations stemming from nanomedicine. The animal models did not change before and after the nanomedicine fever. But somehow, we expect a better translation.

## The Danger of Hypes: History rhymes

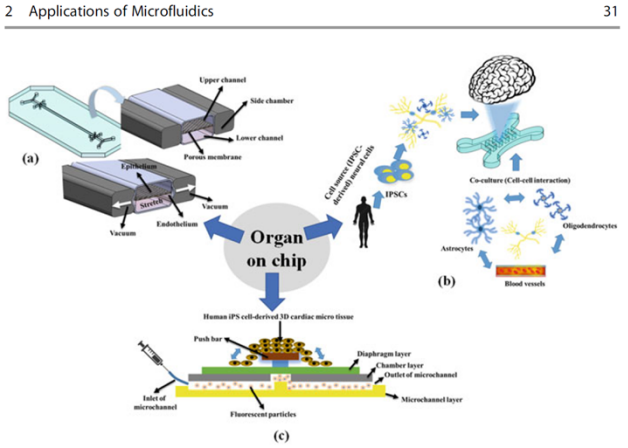
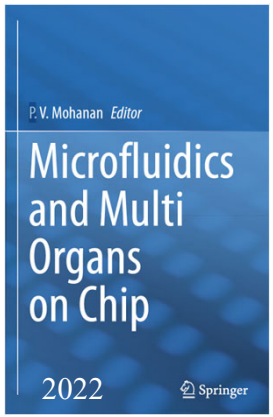



**Fig. 2.2** The organ-on-chip models: (**a**) lung on chip [181], (**b**) brain on chip [195], and (**c**) eye-on-chip [196]

### 2.5.4 Future Scope: Human-on-Chip

Despite significant progress in developing organ-on-chip and micro-engineered tissues, effective drug toxicity testing actually should involve the implementation of each organ and its interactions. Thus, the complete organs must be functionally integrated into the human body in the future, establishing a fully functionalized microfluidic circulatory system. Still, there is a lot of work to be done in the development of complex and complete models that recapitulate the metabolism and physiology of the entire organs. Thus, researchers have further put forward the concept of "human-on-chip" models by interconnecting individual chambers composed of the whole organ model. Each compartment contains different cell types



# Let's Talk about Real Problems

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SYSTEMATIC REVIEW

WIREs NANOMEDICINE AND NANOBIOTECHNOLOGY WILEY

## Current landscape of treating different cancers using nanomedicines: Trends and perspectives

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**Abstract**

The efforts to use novel nanotechnologies in medicine and cancer have been widespread. In order to understand better the focus areas of cancer nanomedicine research to date, we conducted a survey of nanomedicine developmental and clinical research in conjunction with treatment of various cancers. The survey has been performed based on number of publications, rate of citations, entry into clinical trials, and funding rates by the National Cancer Institute. Our survey indicates that breast and brain cancers are the most and one of the least studied by nanotechnology researchers, respectively. Breast cancer nano-therapies seem to also be most likely to achieve clinical translation as the number of publications produced, amount of funding, total citations, and clinical trials (active and completed) are the highest when compared with research in other cancers. Brain cancer, despite its low survival, has capture much less attention of nanomedicine research community as survey indicated, although nanotechnology can offer novel approaches which can address brain cancer challenges.

This article is categorized under:

Therapeutic Approaches and Drug Discovery > Nanomedicine for Oncologic Disease

**KEYWORDS**

animal models, brain cancer, breast cancer, cancer types, clinical trials, nanomedicine, nanoparticles, nanotherapeutics, translation

### 4.1 | Major biological barriers limiting brain cancer treatment efficacy

The BBB, BTB, and tumor microenvironment are major biological challenges in brain cancer research and treatment. As it is known, the BBB is one of the main obstacles to effectively treating primary and metastatic brain tumors as many therapeutics delivered systemically cannot penetrate the BBB because of the tight junctions between capillary endothelial cells. Currently, several drug-based and device-based methods can cause a transient disruption of the function and structure of the BBB. The drug-based category includes Mannitol (Rapoport, 2001), RMP-7 (Bartus et al., 1996), and Regadenoson (Jackson et al., 2016). The second category comprises focused ultrasound technique paired with microbubbles (Jones & Hynynen, 2019), cranial-implantable ultrasound (Idbaih et al., 2019), and MRI-guided focused ultrasound (Kinoshita et al., 2006). Further optimization of these techniques over time may increase the use of nanoparticles in brain cancer treatment. Regardless of the significant technical advances in manipulating the BBB, it would be important to continue investigating the possible morphological and physiological side effects that the transient BBB's disruption might induce and how reversible this disruption is. Kovacs et al. reported that opening the BBB with focused ultrasound causes a sterile inflammatory response (SIR) in the parenchyma. The SIR is compatible with ischemia or mild traumatic brain injury (Kovacs et al., 2017). Additional adverse effects may include neuronal dysfunction, inflammation, and degeneration because of the leakage of membrane proteins, entry of toxins or pathogens into the CNS, the release of cytokines, and an imbalance of ions and transmitters (Profaci et al., 2020).

The other major barrier in brain cancer is the BTB, characterized by an abnormal pericyte distribution and loss of astrocytic end-feet and neuronal connections (Arvanitis et al., 2020). The BTB limits chemotherapeutic efficacy and

### 4.2 | Lack of suitable animal models for brain cancer

The development of brain cancer animal models is complex due to the difficulty of recapitulating BBB (Wiley et al., 2013). For example, a common animal model for high-grade gliomas (HGG) is the intracranial xenograft model in which U87, U251, T98G, and A172 HGG cell lines are often used (Kijima & Kanemura, 2017). Although this model offers some advantages, such as high engraftment success, acceptable reproducibility, and reliable tumor growth and progression, also it presents some limitations, including the lack of single-cell invasion and faithfully recapitulation of the vascular characteristics of the majority of HGG patients (Huszthy et al., 2012; Radaelli et al., 2009). A further comprehensive characterization of the BBB and its interaction with the drug delivery systems is essential to test new therapeutics for brain cancers. At the same time, the use of GEMM and orthotopic PDX for brain cancer research is preferable over xenograft models (Aldape et al., 2019). Other scientific and clinical limitations to addressing brain cancer tumors include a monotherapy approach (i.e., applying a single treatment in the animal-treated group). In clinical practice, human brain tumors are often treated with sequential treatments that involve surgery, radiotherapy, and chemotherapy. Thus, a better alignment of clinical practice recommendations and preclinical experimental design may improve translational efforts.

### 4.3 | Brain cancer clinical trials based on nanotechnology approaches

Currently, there is a low number of nanotechnology-related clinical trials for the treatment of brain cancer. Main benefits of nanoparticles' use in brain cancer treatment are the improvement of drug solubility and potential for more effective drug transport across BBB. Encapsulating drugs in nanobase carriers improves their solubility and allows for more effective delivery using convection-enhanced delivery (CED). Clinical trial Phase I and II studies investigated the side effects of panobinostat nanoparticle formulation termed MTX110 in treating patients with newly diagnosed diffuse intrinsic pontine glioma (DIPG) (Mueller et al., 2023). The MTX110 is composed of panobinostat, an active ingredient combined with several excipients such as hydroxypropyl-β-cyclodextrin, sodium citrate dihydrate, and citric acid to improve their solubility. The improved solubility of panobinostat enables the effective administration of MTX110 via intratumoral CED. The clinical trial study enrolled seven patients diagnosed with DIPG, and the safety and tolerability aspects of MTX110 were assessed for up to 24 months (Mueller et al., 2023). The results suggest that this nanoformulation was safe and tolerable for enrolled patients. Another Phase I trial was conducted to assess the side effects and identify the best dose of polysiloxane gadolinium-chelates-based nanoparticles (AGu1X) given concurrently to the whole brain radiation therapy for the treatment of multiple brain metastases (Verry et al., 2019).

The lack of nanotechnology-based clinical trial studies for brain cancer may also be due to several pitfalls, including the limited effectiveness of receptor-mediated transcytosis (RMT), a mechanism believed to be responsible for nanoparticle-based drug transport across BBB. Several receptors used as targeting ligands are expressed in the brain endothelial cells aiding in transport across the BBB including the transferrin receptor (TfR), insulin receptor, low-density lipoprotein receptor, and single domain antibodies (sdAbs) (Pulgar, 2018). However, TfR, the most frequently used has some limitations, including lack of brain target specificity and low drug uptake in the brain (Pulgar, 2018).

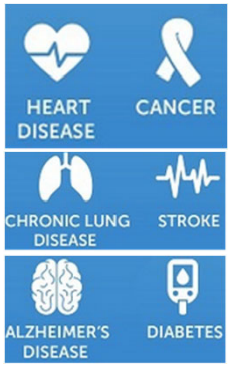
# Future Hurdles of Drug Delivery Systems

2020 — 2030 — 2040 — 2050


**Accurate Testing of Drug Efficacy** → **Key to translation** → **Clinical studies: to evaluate whether a new treatment is "superior", "equivalent", or "non-inferior" to a standard therapy.**

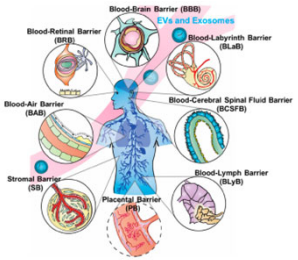
**Long-Term Treatment of Chronic Diseases**

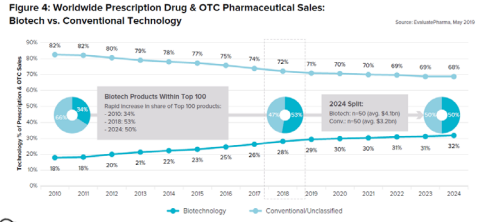
**6/10 adults in the US have a chronic disease and 4/10 adults have two or more.**



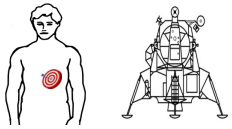


**Overcoming Biological Barriers**

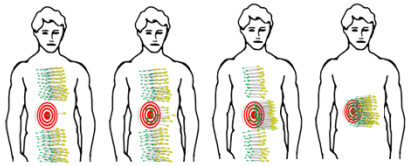


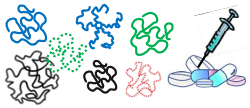




**Delivery of Peptides, Proteins, & Nucleic Acids**



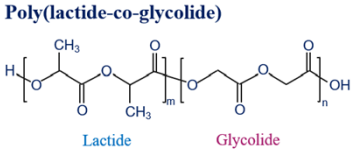
**Delivery with Reduced Side Effects**





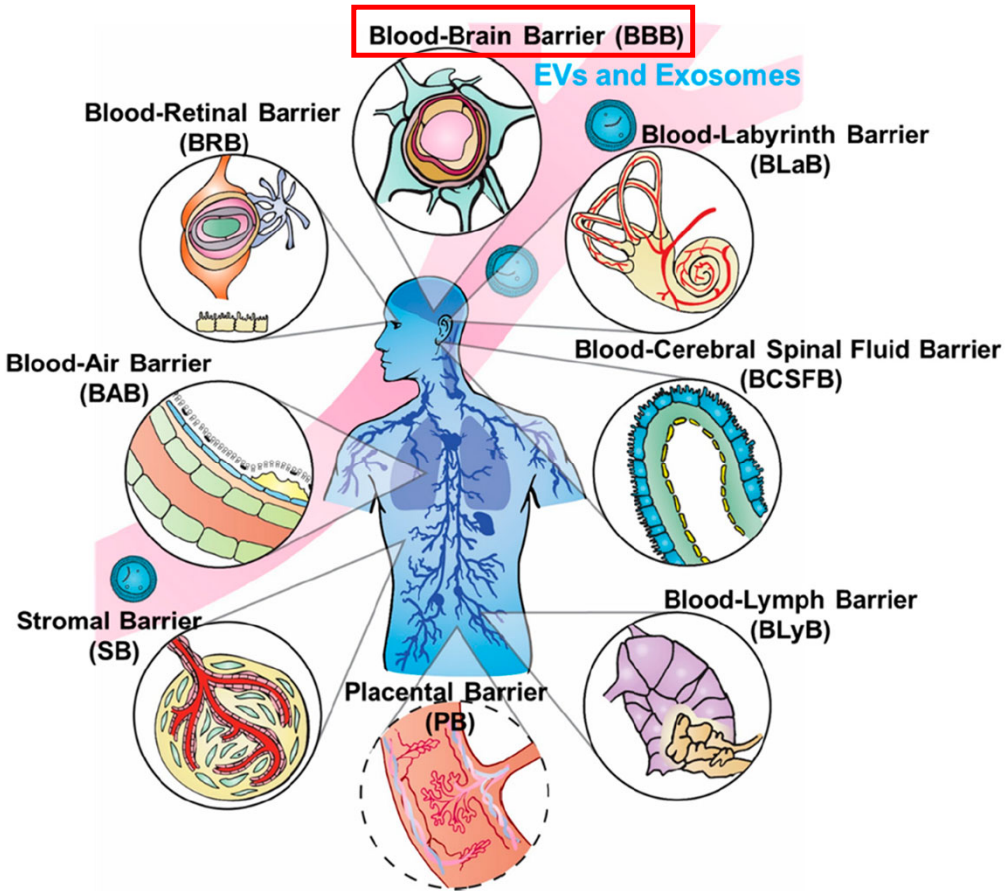
**Development of New Delivery Technologies** → **New Excipients**

→ **Regulatory Risk**


**Health Equity**

# Overcoming Biological Barriers




Eiliott 2021, Unlocking the power of exosomes for crossing biological barriers in drug delivery


Biochemistry

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## Lipid Nanoparticles Deliver mRNA to the Brain after an Intracerebral Injection

Jan Tuma,[▽] Yu-Ju Chen,[▽] Michael G. Collins, Abhik Paul, Jie Li, Hesong Han, Rohit Sharma, Niren Murthy, and Hye Young Lee*

Cite This: https://doi.org/10.1021/acs.biochem.3c00371 Read Online

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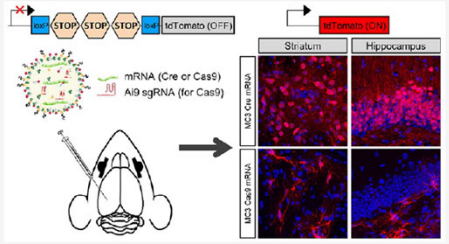
**ABSTRACT:** Neurological disorders are often debilitating conditions with no cure. The majority of current therapies are palliative rather than disease-modifying; therefore, new strategies for treating neurological disorders are greatly needed. mRNA-based therapeutics have great potential for treating such neurological disorders; however, challenges with delivery have limited their clinical potential. Lipid nanoparticles (LNPs) are a promising delivery vector for the brain, given their safer toxicity profile and higher efficacy. Despite this, very little is known about LNP-mediated delivery of mRNA into the brain. Here, we employ MC3-based LNPs and successfully deliver Cre mRNA and Cas9 mRNA/Ai9 sgRNA to the adult Ai9 mouse brain; greater than half of the entire striatum and hippocampus was found to be penetrated along the rostro-caudal axis by direct intracerebral injections of MC3 LNP mRNAs. MC3 LNP Cre mRNA successfully transfected cells in the striatum (~52% efficiency) and hippocampus (~49% efficiency). In addition, we demonstrate that MC3 LNP Cas9 mRNA/Ai9 sgRNA edited cells in the striatum (~7% efficiency) and hippocampus (~3% efficiency). Further analysis demonstrates that MC3 LNPs mediate mRNA delivery to multiple cell types including neurons, astrocytes, and microglia in the brain. Overall, LNP-based mRNA delivery is effective in brain tissue and shows great promise for treating complex neurological disorders.




Tuma 2023, Lipid nanoparticles deliver mRNA to the brain after an intracerebral injection

# How to Improve Animal Models for Better Cancer Treatment?

**It's Not the Animal Model, Inadequate.**
**It's the Human Use, Inadequate.**

Much of the published animal data on nanomedicine is irrelevant to clinical translation.

- Our interpretation of the animal data is often too optimistic.
- Most animal data are presented in a highly positive way to increase their values.
- Only positive results of animal studies are published.
- One common manifestation of cancer nanomedicine is the use of saline solutions as a control.

Publishing negative results is very difficult, making animal models seemingly unsuitable for studying cancer nanomedicine.

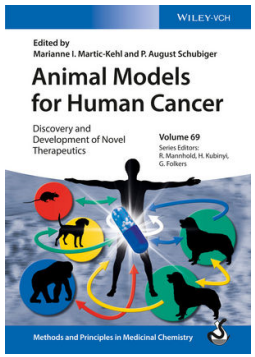
5

## How to End Selective Reporting in Animal Research

*Gerben ter Riet and Lex M. Bouter*

### 5.1 Introduction

Would scientific progress not be a lot swifter and cheaper if we published, in some convenient format, all results from our negative studies too? Although convincing evidence is not available, we think the answer would be affirmative. New empirical results appear daily, but it can sometimes take years for *knowledge* to emerge. Isolated studies may be important, but almost all deeper scientific insights evolve at the meta-level; that is, at the level of collections of similar studies around a particular scientific question. Since the 1980s, in clinical medicine and public health, systematic reviews (often including a meta-analysis) of the literature have been increasingly employed to produce ("meta-level") *knowledge* [1]. These systematic reviews ought to be updated when a new piece of evidence comes along. The crucial role of integration of new findings with existing ones is not always appreciated in animal experimental work, although its justification was eloquently expressed over a century ago:




How to End Selective Reporting in Animal Research
Gerben ter Riet and LexM. Bouter
(Martic-Kehl 2016, Animal Models for Human Cancer)