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

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.

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

<table>
<thead>
<tr>
<th>Term</th>
<th>Introduced in</th>
<th>FDA Approval</th>
<th>Formulation</th>
<th>Nanomedicine?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liposomes</td>
<td>1964</td>
<td>Doxil (1995)</td>
<td>PEGylated liposome</td>
<td>No→Yes</td>
</tr>
<tr>
<td>Polymer Micelles</td>
<td>1965</td>
<td>Taxol (1992)</td>
<td>Polymer micelle (Cremophor EL)</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Taxotere (1996)</td>
<td>Polymer micelle (Polysorbate 80)</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Onpattro (2018)</td>
<td>PEGylated Lipid Nanoparticle</td>
<td>Yes</td>
</tr>
<tr>
<td>PEGylation</td>
<td>1977</td>
<td>Adagen (1990)</td>
<td>PEGylated protein</td>
<td>Yes</td>
</tr>
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</table>
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.

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>
<thead>
<tr>
<th>Tissue/organ</th>
<th>Neocarzinostatin</th>
<th>Smans</th>
<th>Ovomucoid</th>
<th>Bovine albumin</th>
<th>Mouse albumin</th>
<th>Mouse IgG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor</td>
<td>0.82</td>
<td>0.75</td>
<td>0.6</td>
<td>4.92</td>
<td>4.0</td>
<td>4.71</td>
</tr>
<tr>
<td>Blood</td>
<td>0.35</td>
<td>0.24</td>
<td>0.28</td>
<td>0.86</td>
<td>0.49</td>
<td>0.24</td>
</tr>
<tr>
<td>Liver</td>
<td>10.46</td>
<td>12.25</td>
<td>15.12</td>
<td>24.22</td>
<td>50.71</td>
<td>42.92</td>
</tr>
<tr>
<td>Kidney</td>
<td>4.78</td>
<td>4.48</td>
<td>3.79</td>
<td>7.77</td>
<td>6.6</td>
<td>4.24</td>
</tr>
<tr>
<td>Spleen</td>
<td>6.29</td>
<td>8.9</td>
<td>9.57</td>
<td>24.21</td>
<td>40.40</td>
<td>36.4</td>
</tr>
<tr>
<td>Lung</td>
<td>0.68</td>
<td>0.91</td>
<td>0.41</td>
<td>2.36</td>
<td>2.98</td>
<td>2.13</td>
</tr>
<tr>
<td>Heart</td>
<td>0.42</td>
<td>0.51</td>
<td>0.36</td>
<td>1.25</td>
<td>1.58</td>
<td>1.70</td>
</tr>
<tr>
<td>Stomach</td>
<td>0.10</td>
<td>0.08</td>
<td>0.08</td>
<td>0.48</td>
<td>0.39</td>
<td>0.57</td>
</tr>
<tr>
<td>Duodenum</td>
<td>0.70</td>
<td>0.7</td>
<td>1.6</td>
<td>1.2</td>
<td>1.3</td>
<td>1.2</td>
</tr>
<tr>
<td>Brain</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.03</td>
<td>0.01</td>
<td>0.04</td>
</tr>
<tr>
<td>Skin</td>
<td>0.59</td>
<td>0.47</td>
<td>0.39</td>
<td>0.91</td>
<td>1.29</td>
<td>0.93</td>
</tr>
<tr>
<td>Muscle</td>
<td>1.1</td>
<td>1.1</td>
<td>0.9</td>
<td>0.9</td>
<td>1.7</td>
<td>1.2</td>
</tr>
</tbody>
</table>

We previously used smanses dissolved in lipid contrast medium and showed a marked retention of lipid (a T/B of 1200) in the tumor when we administered smans/limipidol via a tumor-feeding artery (21). This, as a consequence, resulted in unprecedented clinical benefit with few side effects for patients with hepato 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 hyperemia, enhanced permeability, little recovery from lymphatics, and perhaps an architectural uniqueness at the neovascular 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 tumorigenicity of smanses and γ-emitting metal citrates used in radionuclide therapy 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, 90,000) (25); the radioactive transferrin tends to accumulate more in the tumor. The highly tumorigenic properties of macromolecular anticancer agents as seen with smans suggest a direction for the future development of new anticancer agents based on this prototype drug.

Fig. 1. Plasma clearance and intratumor accumulation of various 51Cr-labeled 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. O, NCS (M, 12,000); □, smans (M, 16,000); ■, ovomucoid (M, 29,000); △, BSA (M, 69,000); ▼, mouse serum albumin; ▽, mouse IgG (M, 160,000). Radioactive proteins were injected i.v. at time zero. Values are based on radioactivity. See text for detail.


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

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 chemotherapy 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.

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).

"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 overexpression of receptors on cancer cells is actually not related to the increase in the number of nanoparticles to the tumor site [141].

identification of a problem

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

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

Elaine A. Ostrander, Ph.D.
Chief & NIH Distinguished Investigator
Cancer Genetics and Comparative Genomics Branch
Let's Talk about Real Problems

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

Carolina Salvador Morales  Piotr Grodzinski

Abstract
The efforts to use novel nanotechnologies in medicine and cancer have been widespread. In order to better focus the 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 captured 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 Minimally (Raapoot, 2001), RMD-7 (Barua et al., 1996), and fregadanos (Jong et al., 2016). The second category comprises focused ultrasound technique paired with microbubbles (Jones & Hynynen, 2019), cranial-implantable ultrasound (Aubuh et al., 2019), and MRI-guided focused ultrasound (Kimchi 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 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 (Prendi et al., 2020). The other major barrier in brain cancer is the BTB, characterized by an abnormal pereyve tissue distribution and loss of astrocytic endfeet and neuronal connections (Amadori 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, PC9, and A52 E1H2G cell lines are often used (Kajbje et al., 2017). Although this model offers some advantages, such as high engraftment success, acceptable reproducibility, and reliable tumor growth and progression, it also presents some limitations, including the lack of single-cell invasion and faithfully recapitulation of the vascular characteristics of the majority of HGG patients (Hausler et al., 2012; Raddell et al., 2009). A further comprehensive characterization of the BBB and its interaction with the drug delivery systems is essential to test new therapies for brain cancers. At the same time, the use of GEMM and orthotopic PDXA for brain cancer research is preferable over xenograft models (Adalpe et al., 2019). Other scientific and clinical limitations in addressing brain cancer tumors include a more heterogeneous approach (i.e., applying a single treatment in the animal-treated group). In clinical practice, human brain tumors are often used with sequential treatments that involve surgery, radiotherapy, and chemotherapy. Thus, a better alignment of clinical practice recommendations and preclinical experimental design may improve translation 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 nanomedicines in brain cancer treatment are the improvement of drug solubility and potential for more effective drug transport across BBB. Encapsulating drugs in nanoscale carriers improves their solubility and allows for more effective delivery using connection-enhanced delivery (CED). Clinical trial Phase I and II studies investigated the side effects of paclitaxel nanoparticle formulation known as MX310 in treating patients with newly diagnosed diffuse intrinsic pontine glioma (DIPG) (Muehler et al., 2023). The MX310 is composed of pemetrexan, an active ingredient combined with several excipients such as hyaluronic acid/polyethylene, sodium citrate-dihydrate, and starch acetate to improve their solubility. The improved solubility of pemetrexan enables the effective administration of MX310 in intravenous CED. The clinical trial study enrolled seven patients diagnosed with DIPG, and the safety and tolerability aspects of MX310 were assessed for up to 24 months (Muehler et al., 2023). The results suggest that this nano formulation was safe and tolerable for enrolled patients. Another Phase I trial was conducted to assess the side effects and identify the best dose of polyethylene glycol-diol-based nanoparticles (AGU14X) given concurrently to the whole brain radiation therapy for the treatment of multiple brain metastases (Vory et al., 2019). The lack of nanomaterial-based clinical trial studies for brain cancer may also be due to several pitfalls, including the limited effectiveness of receptor-mediated nanoparticles (RMTI), 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 (TIR), insulin receptor, low-density lipoprotein receptor, and single domain antibodies (sAbN). (Pulgar, 2013). However, 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

- **Accurate Testing of Drug Efficacy**
- **Long-Term Treatment of Chronic Diseases**
- **Overcoming Biological Barriers**
- **Delivery of Peptides, Proteins, & Nucleic Acids**
- **Delivery with Reduced Side Effects**
- **Development of New Delivery Technologies**
- **Health Equity**

**Clinical studies:** To evaluate whether a new treatment is “superior”, “equivalent”, or “non-inferior” to a **standard therapy**.

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

**Key to translation**

**Regulatory Risk**
Overcoming Biological Barriers

Blood-Brain Barrier (BBB)
Blood-Retinal Barrier (BRB)
Blood-Labyrinth Barrier (BLaB)
Blood-Cerebral Spinal Fluid Barrier (BCSFb)
Blood-Air Barrier (BAB)
Stromal Barrier (SB)
Placental Barrier (PB)

Biochemistry

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

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/A9 sgRNA to the adult A9 mouse brain, greater than half of the entire stratum 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/A9 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.

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

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.